SCIENTIFIC PROCEEDINGS.

VOL. XXV. APRIL, 1928. No. 7.

Minnesota Branch.

University of Minnesota Medical School,
February 29, 1928, and March 27, 1928.

3926

Studies on Folin Method of Analysis for Glucose in Normal Urine.

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Folin stated[1] that acid caused some interfering substance to dissolve from the Lloyd's reagent used in the determination of glucose in normal urine. It seemed that, if acid caused the interfering substance to dissolve, the Lloyd's reagent might first be treated with acid and this substance removed. Accordingly, some of the reagent was treated for some time with concentrated hydrochloric acid. The acid was diluted and filtered off, the Lloyd's reagent was sucked free of liquid, washed thoroughly, dried at 110° C., and powdered in a mortar. The reagent thus treated, while giving lower reducing values than the untreated reagent, still did not allow of quantitative recovery of added glucose. The reagent had changed in color to a greenish tinge, and the acid filtrate contained much iron. It was then treated similarly with concentrated nitric acid, when the color changed to a light gray—almost white. The latter product allowed of quantitative recovery of added glucose, without the use of permutite, and gave values that were somewhat lower than those obtained by the use of the untreated Lloyd's reagent with permutite. Powdering the permutite caused an increase of 5 or 6 mg. % in the

[1]Folin, Otto, and Svedberg, Andrea, *J. Biol. Chem.*, 1926, lxx, 405.

These results can be best explained if one assumes that the ionized portion is not antiseptic. In the above solution the germicides and the buffers were mixed just before they were tested. All the buffers used contained the same molecular concentration of salts so that the osmotic pressures of all the germicidal solutions used in the above table were approximately the same. This shows that the effects observed could not be due to differences in osmotic pressure.

This is further substantiated by the next experiment where the germicidal solutions were allowed to age before they were tested. The fact that they lost in germicidal power on ageing cannot be explained by any osmotic effects, or by any change of the organisms that are placed in the germicidal solutions. It would appear that the formation of the salts of these compounds requires time and for that reason the germicidal value of these solutions on the alkaline side would decrease on ageing. The following data show that such is the case:

TABLE II.

Effect of ageing upon the germicidal efficiency of alkaline phenol solutions.

Antiseptic	No buffer	In 2% Na_3PO_4 fresh	In 2% Na_3PO_4 24 hours old
1-80 Phenol	0	800	2400
3% Resorcin	0	0	1000

Similar evidence can be obtained from a study of the antiseptic properties of the basic and acid dyes. Burke and Grieve[5] have shown that the basic dyes are rather weak germicides in acid media but are strong germicides in basic media. These substances will form salts in acid media which should ionize rather well, whereas in basic media they will act like weak bases which ionize poorly. The acid dyes on the other hand behave in the same way as phenol.

If the unionized molecule is the one that penetrates the cell it should be possible to increase the germicidal properties by the addition of common ions and to decrease the antiseptic power by the addition of other ions. Such experiments, however, must be interpreted with extreme caution, as the effect of mass action may interfere as described below. The following data will illustrate this point.

Here all the salts added increased the germicidal efficiency. If one confined his attention to the NaCl he could claim that the common ion depressed the ionization of sodium phenate and thereby increased the germicidal efficiency, and the other salts would appear to give contradictory evidence. One must bear in mind here, however, that a very large amount of the various salts were added in

[5] Burke and Grieve, *Am. J. Med. Sci.*, clxviii, 98.

TABLE III.

Effect of salts on the germicidal efficiency of aromatic compounds.

Antiseptic	Salt added	Surviving bacteria	
2.5% Resorcin	2% NaCl	75	pH 10
.1% B. Naphthal	"	420	"
Hexyl Resorcin (1-10,000)	"	450	"
2.5% Resorcin	No salt	1200	"
.1% B. Naphthal	"	10000	"
Hexyl Resorcin (1-10,000)	"	900	"
Phenol (1-100)	"	450	"
"	2% NaCl	0	"
"	3.8% $CaCl_2$	0	"
"	1.9% NH_4Cl	0	"
"	2.5% KCl	0	"
"	No salt	135	pH 7

comparison to the molar content of sodium phenate present. It appears that the law of mass action would come into play here so that in the case where the KCl was used, the potassium would replace the sodium in the sodium phenate. On this basis one would be justified in calling it a common ion effect in every case of the above table. An identical result would be obtained if one added the same amount of potassium phenate to the solution as there was sodium phenate in the former case and then added the potassium chloride and a small amount of sodium chloride. In this case one would certainly be justified in calling it a common ion effect. The phenal certainly would not know which one of these salts were added first.

To show the effect of added electrolytes that do not furnish a common ion, one would have to use a small amount of the electrolyte in comparison to the amount of the germicide used. In this connection we find that small amounts of KCl added to sodium phenate decrease the germicidal power, whereas NaCl increases it. Tilley and Schaffer[6] found that NaCl added to NaOH and actiseptic sodium salts increased their germicidal activity. A similar effect has also been reported by Levine, Toulouse and Buchanan.[7] They find that NaCl, Na_3PO_4 and Na_2CO_3 will increase the germicidal power of NaOH and they suggest that it is the unionized NaOH which penetrates the cell, and that the addition of common ions depress the ionization. Their data show that for equivalent amounts of sodium added these 3 substances have the same quantitative effect. We have found similar effects of NaCl on Na_3PO_4 and we find further that KCl, NH_4Cl, $CaCl_2$, which do not furnish a common

[6] Tilley and Schaffer, *J. Infect. Dis.*, xxvii, 358.

[7] Levine, Toulouse and Buchanan, *J. Ind. and Eng. Chem.*, xx, 179.

ion decrease the germicidal efficiency. The effect of the KCl, however, is very slight. Since fairly high concentration of Na_3PO_4 has to be used to get any germicidal effect, fairly large amounts of the other electrolytes can be added in this case without obtaining the effect of the mass action as was experienced with the phenol.

Since *Staphylococcus aureus* is very difficult to kill by the phosphates alone, we used for some of these experiments young cultures of *Staphylococcus auranticus,* a less resistant organism. If the *Staphylococcus aureus* were used there would be such a large number of surviving organisms that the experimental error in counting would be too great.

The following data illustrate typical results:

TABLE IV. *Effect of electrolytes on germicides.*

Germicide			Organisms surviving			
	No salt	+NaCl 2%	+KCl 2.55%	+NH_4Cl 1.81%	+$CaCl_2$ 3.78%	
8% Na_3PO_4	540	300	630	10,000	10,000	
*2% Na_3PO_4	760	360	450	2,400	10,000	
	No salt	1% NaCl	5% NaCl	1% KCl	5% KCl	.01%KCl
4% Na Phenate	38	18	26	17	60	100

*For this *Staphylococcus aurenticus* was used.

In view of these results we believe that the evidence is in favor of the hypothesis that the concentration of the unionized portion of an antiseptic is the factor of prime importance in determining germicidal efficiency.

3928

An Experimental Study of Plasma Protein Regeneration.

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The object of this paper is to study the restoration of the several fractions into which the plasma globulins and albumins may be divided, after severe bleeding of dogs. No general anesthetic was used in the experiments. The dogs were bled from a large vein either in the hind or front leg. Plasma-pharesis was employed to maintain the cell volume and blood viscosity at a high level.

The fractionating of the plasma proteins was done by various concentrations of sodium sulphate at a pH of 7. The nitrogen in each fraction was determined by the macro-Kjeldahl method and titration carried out with N/20 alkali.

TABLE I—Dog No. 5.
The distribution of the protein fractions in the plasma.
Results expressed as mg. nitrogen in 100 cc.

Date	Weight kg.	Cell vol. %	Total plasma nitrogen	Non-protein nitrogen	Fibrinogen	Euglobulin	Pseudoglobulin	Albumin I	Albumin II	Albumin III	Globulin albumin ratio
7-19	17.5	41.0	1011.6	29.6	86.7	15.5	201.9	102.4	40.9	564.2	2.31
7-21	17.4	37.6	614.5	21.0	75.9	13.6	90.7	60.4	39.1	334.8	2.41
7-22	17.0	30.0	761.2	14.0	51.7	19.6	123.8	76.0	67.3	422.8	2.90
7-26	16.5	24.0	943.2	71.5	160.6	22.4	144.3	182.3	74.2	359.4	1.88
7-29	15.9	26.0	954.2	38.0	161.5	30.2	166.7	164.8	45.8	385.2	1.66

The average results from the successful experiments on six dogs indicated that all the globulin fractions decrease more rapidly during and immediately after bleeding. After a lapse of 3 to 4 days, depending upon the severity of the bleeding, the globulins appear to be restored more rapidly. This shift in regeneration greatly influences the albumin-globulin ratio.

The total protein nitrogen was determined by the macro-Kjeldahl method; the non-protein nitrogen, by the technique of Folin. The fractionation of the proteins into fibrin, euglobulin, pseudoglobulin, and the three albumin fractions was carried out according to an unpublished method of Berglund. The nitrogen in each fraction was determined by a macro-Kjeldahl digestion and titration with N/20 alkali.

Table I is illustrative of the type of result obtained in the experiments.

It is to be clearly understood that the fractions of globulins and albumins shown in Table I are not to be considered as definite protein entities. Hersfeld and Klinger[1, 2, 3] believe that the protein fractions do not have a chemical individuality but are an interrelated series of colloidal particles of different degrees of dispersion, which can be transposed one into the other. In other words, the evolution begins with the lowest dispersed particles, fibrinogen, and extends to the albumins and non-coagulable substances.

Our results seem to be somewhat in accord with this view. It can be said, however, that there seems to be a definite shift in the values toward the globulins as the regeneration takes place. This fact brings about a definite change in the globulin-albumin ratio (G/A). Instead of being 2.5, it changes to 1.6.

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[1] Herzfeld, E., and Klinger, R., *Biochem. Z.*, 1917, lxxxiii, 228.

[2] Herzfeld, E., and Klinger, R., *Biochem. Z.*, 1919, xcix, 204.

[3] Herzfeld, E., and Klinger, R., *Ergebn. Hyg., Bakt., Immunitätsf. exper. Therap.*, 1920, iv, 282.

3929

A Statistical Study of Normal Male Adult Human Hypophysis.

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Due to the uncertainty that still exists with reference to the involvement of particular parts of the hypophysis in certain diseases, and the general lack of quantitative data on the normal proportion of the different subdivisions of this organ, it seemed desirable to study by recognized statistical methods enough normal hypophyses to give standards which would be sufficiently accurate for evaluating abnormal proportions. Clinical and pathological literature contain numerous statements to the effect that one or more parts of the hypophysis are enlarged or are too small, etc., without the author knowing the range of normal variability. In many cases unwarranted clinical deductions are based on such statements. This condition can lead only to further confusion.

The material used consisted of 111 carefully selected normal male hypophyses, 20 to 76 years of age, from cases of relatively sudden accidental death. After getting the fresh weight of the organ serial sections were made. Every 20th section was projected and the different parts of the gland outlined at a magnification of 20 diameters, in general. In certain cases *pars intermedia* was projected at a higher magnification (up to 100 diameters). The different portions of the outline were cut out and weighed. This gave the proportion that any particular part constitutes of the whole. The weight of the paper was always more than 100 times the weight of the corresponding part of the fresh organ. The method is more fully explained in a previous note.[1] The routine method of obtaining the hypophysis at autopsy made it necessary to cut off and discard the stalk and as much of the surrounding connective tissue as possible.

The frequency distribution of the weight of the whole gland, of the anterior lobe, and of the posterior lobe, is of the common fairly symmetrical unimodal type; while that of the epithelium of *pars intermedia* and of the colloid is greatly skewed in the positive direction, *i. e.*, towards the small values.

A summary of the absolute weights (in mg.) follows: The anterior lobe varied from 224 to 611; mean, 394±5.4; standard deviation, 84.5; coefficient of variation, 21.4. The posterior lobe

[1] PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 416.

varied from 27 to 246; mean, 121±2.3; standard deviation, 35.7; coefficient of variation, 29.1. The epithelium of *pars intermedia* varied from .5 to 20.1; mean, 4.6±.3; standard deviation, 4.3; coefficient of variation, 93.9.

These figures thus give quantitative support to the great variability of *pars intermedia* as frequently noted from general inspection of a limited number of sections. While some argue that there is no true functional *pars intermedia* in the adult human hypophysis —the epithelium in this region being merely a rudimentary structure or, according to others, an invasion from the anterior lobe— it would seem best to retain the term *pars intermedia* for the epithelium and associated colloid in the usual location for *pars intermedia*. However, it may be so small in a normal human adult as to be equivalent to a cube only .8 mm. in dimensions and on the average would make a cube slightly less than 1.7 mm. in size. These small quantities should be kept in mind by those who ascribe much functional importance to this part of the hypophysis.

The colloid associated with *pars intermedia* and within the residual lumen varied from 0.1 to 51.1; mean, 6.3±6; standard deviation 8.6; coefficient of variation, 137. There is absolutely no correlation between the amount of colloid and the amount of epithelium in *pars intermedia*.

The whole gland (without capsule, stalk and *pars tuberalis*) varied from 358 to 788; mean, 526±6.2; standard deviation, 96.8; coefficient of variation, 18.4. The fresh gland after being dissected out (*i. e.*, including on the average 44.5 mg. of the surrounding connective tissue or capsule that could not be dissected away without tearing the gland, but without stalk or *pars tuberalis*) varied from 400 to 855 and averaged 570.

The relative weights of the different parts of the hypophysis as percentage of the whole gland (with capsule, stalk and *pars tuberalis*) are as follows: Anterior lobe varied from 56 to 92; mean, 74.8±0.4. Posterior lobe varied from 7.1 to 41.3; mean, 23.1±0.4. The epithelium of *pars intermedia* varied from 0.13 to 3.64; mean, 0.85± 0.05. The colloid in the region of *pars intermedia* and in the residual lumen varied from 0.02 to 10.39; mean, 1.20±0.10.

The correlation coefficient (r) between the weight of the hypophysis and stature is +0.28±0.06. This is entirely due to the anterior lobe. In the case of the weight of the anterior lobe and stature, r = +0.31±0.06. There is a moderate and significant negative correlation between the weight of the anterior lobe and age (r = −0.29±0.06). This decrease in the anterior lobe takes place mostly after 50 years of age. There is probably a small positive

correlation between the weight of the posterior lobe and age ($r = \pm 0.22 \pm 0.06$). Hence in the case of the whole gland, the correlation with age is small, negative, and only of probable significance ($r = -0.19 \pm 0.06$).

3930

Ruffed Grouse Are Susceptible to Tularemia.

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The diminution in number of wild rabbits and ruffed grouse has occurred simultaneously in Minnesota during the last 4 years. Human cases of tularemia have occurred in Minnesota during the same period. The occurrence of specific agglutinins in the blood of many of the wild rabbits still remaining has been indicated by studies which we are carrying out in Hubbard County. This finding points to tularemia as the cause of the disappearance of the rabbit. The susceptibility of the ruffed grouse to tularemia has been studied, as it was felt that there was some relationship between the disappearance of grouse and the disappearance of the rabbit. At the outset of the work it was found that Parker and Spencer[1] had considered the possibility of grouse susceptibility, and produced an infection in blue grouse. They demonstrated that the rabbit tick *Haemaphysalis leporis-palustris* is an important carrier of tularemia, and further pointed out that this parasite is found on both rabbits and game birds. There is, therefore, a possible natural carrier of the disease between rabbits and grouse.

The grouse used in our transmission experiments were ruffed grouse *(Bonasa umbellus)* and were trapped wild in Hubbard County. Before inoculation they were tested for agglutinins and their blood did not agglutinate *Bact. tularense antigen* in dilutions of 1-10 or above.

The strain of *Bact. tularense* used for inoculation was obtained from a human case of tularemia originating in Hubbard County from the bite of a deer-fly. The organism was grown in pure culture on rabbit blood-cystin agar. At the beginning of this work it was inoculated into a guinea pig, which died on the 3rd day with

[1] Parker, R. R., and Spencer, R. R., Sixth Biennial Report of the Montana State Board of Entomology, 1925-1926, 30.

liver and spleen studded with nodules. Material from this guinea pig was used to inoculate grouse No. 1.

Grouse No. 1. Inoculated subcutaneously and intramuscularly with spleen emulsion. The bird was dead on the morning of the 3rd day. There was slight infiltration of tissue at the site of inoculation. The peritoneal cavity contained about 3 cc. of slightly bloody fluid. All internal organs appeared normal macroscopically. Material was inoculated into 4 guinea pigs as follows:

No. 1. Peritoneal fluid. Dead on 2nd day without typical lesions. An exudate was present over abdominal wall which was inoculated into another guinea pig, which died with typical lesions of tularemia.

No. 2. Spleen. Died on 2nd day. Inguinal glands enlarged and exudate over abdominal wall similar to No. 1.

No. 3. Heart's blood. Dead on 3rd day. Enlarged nodes, spleen and liver typical of tularemia.

No. 4. Emulsion of breast muscle. Died on 3rd day. Enlarged nodes. Spleen and liver typical of tularemia.

Two control guinea pigs were inoculated at the same time and with the same material as grouse No. 1. Both died on the 3rd day with lesions typical of tularemia.

Grouse No. 2. Inoculated subcutaneously with spleen from guinea pig which had been inoculated with heart's blood from grouse No. 1. Grouse No. 2 died on the 9th day. No lesion formed over site of injection. Liver and spleen did not show any lesion grossly. All internal organs appeared normal macroscopically. Three guinea pigs were inoculated subcutaneously with material from grouse No. 2, as follows:

No. 1. Heart's blood. Died on 4th day. Inguinal glands, liver and spleen typical of tularemia.

No. 2. Spleen and kidney. Died on 4th day. Inguinal glands enlarged. Liver only slightly involved. Spleen typical.

No. 3. Liver. Died on 4th day. Inguinal and axillary glands enlarged. Spleen typical.

Grouse No. 3. Inoculated by abrasion through skin of back with dilute heart's blood of grouse No. 2. Grouse No. 3 died on the 6th day. No definite ulceration or gross expansion of the initial lesion was noticeable. Liver and spleen showed no nodules. All internal organs appeared normal macroscopically. Four guinea pigs were inoculated subcutaneously with material as follows:

No. 1. Heart's blood. Died on 5th day. Inguinal and axillary nodes enlarged, spleen and liver typical of tularemia.

No. 2. Spleen. Died on 4th day. Inguinal glands enlarged. Spleen typical.

No. 3. Liver. Died on 5th day. Inguinal glands enlarged and spleen typical.

No. 4. Lung. Died on 5th day. Right inguinal and right axillary glands involved. Spleen typical of tularemia.

It would appear that the ruffed grouse succumbs to an experimental infection with *Bact. tularense* with the same regularity as the guinea pig and the rabbits. There appears to be little tendency for the formation of a local lesion. Although guinea pigs and rabbits usually develop macroscopic lesions by the 4th day, no lesions were visible in the grouse which died even as late as the 6th and 9th day. That the organism produces a true septicemia is indicated by its wide distribution in the body and its isolation from normal appearing tissues, such as lung. It would appear that the occurrence of tularemia in grouse as a natural disease is a probability. They are highly susceptible to the disease and subject to infestation by a natural insect carrier, common, according to Parker and Spencer, to grouse and rabbits. If the disease does occur as a natural infection in grouse, its presence might easily be overlooked at necropsy by the absense of gross lesions of the disease. It appears that no well defined human cases of tularemia have been reported as the result of cleaning grouse. This would not appear to invalidate the possibility of the natural infection of grouse, as few such birds are killed and cleaned compared with the number of rabbits. The indications justify a diligent search for tularemia in grouse dying from disease. *Bacterium tularense,* already of great importance because of its ability to produce infections in numerous species of mammals, becomes even more remarkable by its ability to produce at least experimental infection in birds.

3931

Significance of Reticulocyte as Index of Regeneration in Different Types of Experimental Anemias.

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A new emphasis has recently been placed on the reticulocyte count of the circulating blood through its introduction by Minot and Murphy as a convenient check of the effects of a liver diet in Pernicious

Anemia. In the untreated cases of Pernicious Anemia during the anemic stage the reticulocyte count is usually less than 1%, and sometimes *nil*. The behavior of the reticulocyte count during a spontaneous remission can hardly be studied now, since one does not feel justified in waiting for such a remission to occur. We have endeavored by the production of different types of anemias to use the reticulocyte count as a measure for the intensity of spontaneous regeneration of erythrocytes. One of the authors has recently pointed out the usefulness, in the consideration of the anemias, strictly to distinguish between the morphological and chemical phases of blood regeneration. In Phenylhydrazine anemia we encounter an anemia due chiefly to the destruction of the erythrocyte with but little loss of the hemoglobin from the body, and as far as we know, without any direct damage to the hemoglobin synthesis in the body.

The second type of anemia which we have employed—destroying erythrocytes by intravenous injections of distilled water—is similar to Phenylhydrazine Anemia in this respect that only a small amount of the liberated hemoglobin is lost through the kidney, the largest amount being disposed of within the organism.

In direct opposition to these 2 types stands the third type of anemia produced by repeated bleeding, in which form the cells are removed and no hemoglobin or hemoglobin products are left in the organism.

Before presenting our experimental data we offer these conclusions to which we have arrived in regard to the interpretation of the reticulocyte count: 3 factors are of importance in estimating the regenerative value of a given reticulocyte count. Neither the percentage of reticulocytes, nor the total number per unit volume is sufficient for a proper interpretation. The further factors for consideration are the level of the erythrocyte count and the direction in which the erythrocyte count is moving, that is, increasing or decreasing. For example, a reticulocyte count of 5% with a total erythrocyte count of 4,000,000 per cubic millimeter, means a rapid regeneration and a total reticulocyte count of 200,000 per cubic millimeter; while a 5% reticulocyte count with an erythrocyte count of 1,000,000 per cubic millimeter means a total reticulocyte count of 50,000 per cubic millimeter, and a comparatively low grade regeneration. This point is a fundamental one. The situation is further complicated as follows: A total erythrocyte count of 2,000,000 will show a certain percentage of reticulocytes, say 20% to 30%, while the blood is in the stage of rapid regeneration, but only about 2% to 5% while the blood is rapidly being destroyed.

Only when these points are taken into consideration is it possible properly to use the reticulocyte count as a measure of regeneration in the different types of anemia.

Our results, broadly speaking, show that in Phenylhydrazine Anemia there is an exceedingly rapid regeneration of the erythrocytes, with reticulocyte counts as high as 800,000 per cubic millimeter or 41.3%, a count similar to that obtained in cases of Pernicious Anemia during the period of maximal response to the feeding of raw liver. This regeneration is so intensive that it requires careful watching to produce a severe Phenylhydrazine Anemia, each injection being followed in 2 or 3 days by intensive regeneration. The danger involved is that as soon as the dosage is pushed hard, one is apt to lose the animal by a too severe anemia or by a consequent intercurrent infection. In some of our experiments, continued over 39 and 49 days respectively, we have not been able to convince ourselves of the possibility materially to slow down the regenerative process by means of a prolonged phenylhydrazine poisoning. The degree of anemia produced was such that the red count for single days was down as low as 680,000 per cubic millimeter, but usually the count was reduced to between 1,000,000 and 2,000,000 per cubic millimeter. The blood picture showed normoblasts reaching a maximum of 11,300 per cubic millimeter. Even in this high count the normoblast always represented a late stage of development with distinctly

TABLE I.

Anemia from intravenous injection of distilled water.

Day of experiment	Water intravenously	Urine		Hemoglobin	Erythrocyte	Reticulocytes	Leukocytes	Normoblasts
		In 24 hours	Remarks					
	cc.	cc.		%				
0	—	—	—	95	5,980,000	5,980=0.1%	12,700	0
1	300	—	—	94	5,870,000	11,740=0.2%	12,700	25
2	920	1200	Bloody	98	6,280,000	6,280=0.1%	13,800	28
5	950	1350	"	64	5,100,000	40,800=0.8%	28,600	29
7	940	1050	"	71	5,440,000	157,900=2.9%	46,600	93
9	675	830	"	59	4,340,000	60,700=1.4%	17,200	34
11	750	1120	"	68	4,640,000	222,600=4.8%	19,300	38
12	720	820	Not bloody	61	4,720,000	184,000=3.9%	28,300	28
13	1025	1190	Bloody	57	4,070,000	289,000=7.1%	29,400	29
14	940	600	Not bloody	52	3,650,000	157,000=4.3%	22,200	44
15	900	660	Bloody	44	3,610,000	122,700=3.4%	36,700	147
16	1230	1050	Bloody	42	2,260,000	149,200=6.6%	17,100	102
18	1520	1670	Very bloody	49	4,750,000	446,500=9.4%	42,600	766
19	—	—	—	50	4,280,000	453,500=10.6%	22,600	158

pycnotic nuclei, and only seldom did the cytoplasm show a marked degree of basophilia. On the whole the reticulocyte curve and the normoblast curve ran parallel. In all cases, the normoblasts were observed in the circulating blood for a long period of time after the anemia had fully disappeared and the reticulocyte count had returned to normal. However, we think it possible that a single normoblast may occasionally be found in the normal dog's circulating blood.

In the production of an anemia by the intravenous injection of distilled water a 16 kg. dog was used. Large amounts of distilled water were required. At first 5 injections of from 600 to 1000 cc. were made, one every 2nd or 3rd day, with a reduction of the erythrocytes from 6,000,000 to 5,000,000. Then a daily injection of from 750 to 1250 cc. of distilled water was given over a period of 6 days, which reduced the erythrocyte count to 2,300,000. The injections were usually followed by a hemoglobinuria and a diuresis. The diuresis must have produced a rather marked dehydration for the drop in the erythrocyte count and hemoglobin estimation did not occur until 1 to 2 days after the injection.

The response of the reticulocytes, erythrocytes and leukocytes was somewhat less intense than in the Phenylhydrazine Anemia. The normoblast response alone was markedly less intense than after phenylhydrazine.

A 15 kg. dog was used for the production of an anemia by repeated bleeding. A total of 1615 cc. of blood was removed in 15 days. This reduced the erythrocytes from 5,200,000 to 2,300,000. The hemoglobin was reduced 15% lower than corresponded to the same erythrocyte count in the other types of anemia. The leukocytes were only slightly affected, if at all. The response of the reticulocytes and normoblasts was much less lively than in the Phenylhydrazine Anemia, and slightly less than in the anemia from distilled water. The maximal reticulocyte count under the most rapid regeneration with 2,300,000 erythrocytes was 310,000 per cubic millimeter, or 13.2%. When compared with the Phenylhydrazine Anemia this is a very mild response; in the latter an erythrocyte count of 2,000,000 corresponded to a reticulocyte count of 476,000, or 23.8%, and an erythrocyte count of 1,100,000 to a reticulocyte count of 438,000 or 38.5%. Regeneration from the posthemorrhagic anemia was complete in 10 to 12 days, which is 1 to 2 days longer than was required for regeneration from a much lower level—980,000 erythrocytes per cubic millimeter in the Phenylhydrazine Anemia. When the anemia produced by distilled water is compared with the posthemorrhagic and with the Phenylhydrazine

Anemia it occupies an intermediate position in its degree of regenerative response.

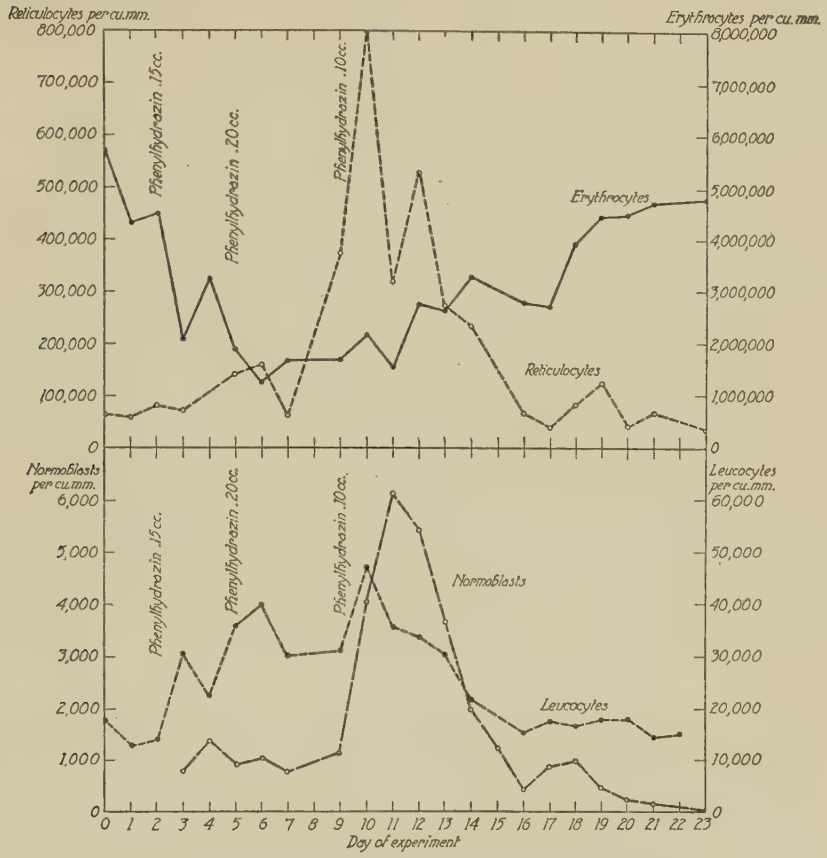

CHART 1. Blood response in Phenylhydrazine Anemia.

3932

Positive Effect of Tyrosine Feeding Upon Excretion of Reducing Urinary Compound in Myasthenia Gravis.

HILDING BERGLUND AND ANNE LOHMANN.

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In the course of the studies of the reducing urinary substance recently described by Medes, Berglund and Lohmann,[1] in a case of

[1] Medes, Grace, Berglund, Hilding, and Lohmann, Anne, PROC. SOC. EXP. BIOL. AND MED., 1927, XXV, 210.

Myasthenia gravis, some feeding experiments with amino acids have been carried out. The subject for our experiments was the same patient with *Myasthenia gravis* which was studied in the publication referred to above. Tyrosine and glycine were fed. Phenyl alanine was not available. The amounts fed were 2.5 gm. and 5 gm. The patient was on a uniform diet and the total amount of reducing power of the urine was determined by the Folin and Berglund[2] method, for the so called normal urine sugar. From previous work it is known that the reducing value of the 24 hour urine in normal individuals on a diet similar to our patient is about 1 gm. of glucose. The urine of our patient, as seen from the table, shows a reducing value corresponding to from 2.5 to 3.0 gm. per 24 hours. As shown in the table, the feeding of tyrosine brings about a definite increase lasting 2 days, while the feeding of glycine shows no significant increase.

Besides the tyrosine experiment reported in the table, tyrosine has been fed to the same patient in other experiments, once in 1 gm. doses continued over 2 days and followed by a 2.5 gm. dose, and once in a 5 gm. dose. In all instances has there been an unmistakable increase in the reducing value of the urine.

Our experiments do not give any information as to the quantitative relationship between the amount of tyrosine fed and the amount of reducing compound excreted. This is for 2 reasons:

TABLE I.

The effect of tyrosine and glycine feeding on the reducing power of the urine in a case of Myasthenia gravis.

Day of experiment	Urine in 24 hours	Creatinine	Reducing value as glucose	Experimental condition
	cc.	g.	g.	
1	2210	1.06	2.51	
2	2035	1.05	2.97	
3	1640	1.02	2.78	
4	2310	1.05	3.41	Tyrosine 5 gm. by mouth
5	1760	1.06	3.55	
6	2060	1.04	2.62	
7	1435	1.05	2.65	
—	—	—	—	
—	—	—	—	
10	2155	1.10	2.64	
11	2305	1.08	2.37	
12	1985	1.05	2.25	
13	1940	1.10	2.42	
14	2585	1.02	2.64	Glycine 5 gm. by mouth
15	2940	1.00	2.12	
16	2240	1.13	2.64	
17	1880	1.11	2.58	

[2] Folin, Otto, and Berglund, Hilding, *J. Biol. Chem.*, 1922, li, 209.

first, the Lloyd treatment of the urine removes some of our reducing compound. In 1 experiment with the isolated and moderately purified compound the first treatment with Lloyd's reagent removed 20 to 30%, a second shaking somewhat less. Second: we do not know how the reducing power of our compound corresponds to the reducing power of glucose. The reduction, as has already been pointed out, is more slow than with glucose. The reduction of the cupric compound when the test is carried out on the urine seems to go on undisturbed, and the final match of the color in the glucose standard and the unknown is perfect. When the purified compound is used the situation is different. Boiling for the ordinary length of time, 8 minutes, then gives on the addition of the phosphomolybdic acid reagent not a blue but a green color. We believe this to be due to the formation of a copper salt of the compound and a direct reaction of this salt with the phosphomolybdic acid.

A direct method for the determination of our compound is wanted but has not been worked out.

Conclusion: The feeding of a cyclic amino acid like tyrosine increases the amount excreted of the reducing urinary compound recently described in a case of *Myasthenia gravis.*

Southern Branch.

Tulane University, February 28, 1928.

3933

Prevention of Peritoneal Adhesions by Use of Vegetable Ferments.*

ALTON OCHSNER AND FRANK MASON. (Introduced by Henry Laurens.)

From the Department of Surgery, Tulane University Medical School.

Many operative technics and the use of numerous substances have been advocated in order to prevent the re-formation of adhesions in patients. All, however, have given about the same negative results. Kubota, in 1924, working with vegetable ferments of the papain group, found that it was possible to prevent the formation of adhesions by introducing vegetable ferments into the peritoneal cavity. Quite recently Buchbinder, using intraperitoneal glucose, and Johnson, using amniotic fluid, report brilliant results in the prevention of peritoneal adhesions.

In order to test the efficiency of the vegetable digestants, the following experimental work was carried out: a series of 39 rabbits and 16 dogs was used, and an attempt made to determine the effect of vegetable digestants on the formation and re-formation of peritoneal adhesions. A peritonitis was produced at laparotomy by rubbing the peritoneum covering the loop of the small bowel with a piece of dry gauze until bleeding occurred. Following this a 7% solution of tincture of iodine was applied over the denuded surfaces.

Series 1. 39 rabbits. At the original laparotomy, and at the time of the production of the peritonitis, solutions of papain or substances of the papain group were introduced into the peritoneal cavity. Animals, used as controls, which had no vegetable digestants added to the peritoneal cavity, as well as those to which the ferment was added, were killed on the 4th, 7th, 10th, 15th days post-

* The major portion of this work was carried out at the University of Wisconsin.

operatively and also 7 weeks after operation. At autopsy the control animals showed evidence of extensive adhesion formation, whereas those treated with the ferment showed either no evidence of adhesion or the adhesions which were formed were fine and veil-like. Control experiments were also carried out using the ferment alone; varying strengths from 1-1000 to 1-400,000 were used, and in none were there any evidences of irritation when papain solution or any of its associated substances were employed. This was studied both macroscopically and microscopically.

Series 2. 16 dogs. The effect of the vegetable digestion on the re-formation of adhesions. In this series a peritonitis was produced as in series 1. Nothing was added to the peritoneal cavity. The animals were allowed to live. Forty-three days later the animals were re-laparotomized and definite peritoneal adhesions found. These were divided. In half of the animals vegetable digestants were added to the peritoneal cavity. This was used in varying strengths. The other half of the animals were used as controls. The animals were sacrificed on the 4th, 7th, 10th, 13th days and 7 weeks after the second laparotomy. Invariably in control animals evidence of numerous adhesions, matting together the loops of the bowels and also extending between the bowel and the parietal peritoneum was found. One animal died of intestinal obstruction due to adhesions. Those animals treated with vegetable digestants in 3 instances showed very fine adhesions, between the operative wound and several loops of intestine, which could easily be broken with the finger. In the remaining dogs no evidence of adhesions was found.

3934

The Treppe in Excitability in the Turtle Ventricle.

RICHARD ASHMAN AND ROBERTA HAFKESBRING.

From the Department of Physiology, Medical School, Tulane University.

In earlier reports, we have demonstrated a treppe in conduction through compressed cardiac muscle, *i. e.,* after a period of quiescence, the conduction times for a series of impulses, more or less rapidly elicited, may show a progressive decrease. In attempting further to elucidate this phenomenon, it became necessary to determine whether, with repeated stimulation, the excitability would

show a similar stepping up. Adrian,[1] in studying the supernormal recovery curve of the frog ventricle, determined the excitability at various intervals following a single response. There is nothing in his paper to show whether, as seemed highly probable, the excitability would have still further increased had there been 2 or more antecedent responses, *i. e.,* whether a treppe of more than 1 step would have appeared.

We placed a freshly excised turtle ventricle in a moist chamber, and recorded the mechanical response while varying the strength of the break induction shock by changing a resistance in the primary. Six turtles were used in these experiments. Using the quiescent ventricle and starting with the least effective stimulus, we found that the effective strength could be progressively diminished when we stimulated repeatedly at intervals varying from 2 to 30 or more seconds. In 1 typical case, when the decrements were small and the interval 2 seconds, the resistance was changed from 25 to 46 ohms before failure of response ensued. On the other hand, with intervals of 30 seconds, the muscle responded only up to 31 ohms. If the decrements were great, *e. g.,* over 0.2 ohm, failure of response appeared sooner because the treppe in excitability did not keep pace with the decrement in strength of stimulus. After a 3 minute rest, the least effective stimulus corresponded to 25 ohms. More prolonged rests, up to 15 minutes, did not result in increased excitability. So far as determined, the changes in mechanical response were closely parallel with those in excitability.

Other findings, as well as evidence that sympathetic stimulation did not cause the results, will be given in a later communication.

3935

Treatment of Ileus by Splanchnic Anesthesia. An Experimental Study.

ALTON OCHSNER, I. M. GAGE AND R. A. CUTTING.
(Introduced by Henry Laurens.)

From the Department of Surgery, Tulane University Medical School.

Markowitz and Campbell[1] have shown that spinal anesthesia will relieve paralytic ileus produced in experimental animals. Believing that in these cases inhibitory impulses were transmitted to the in-

[1] Adrian, E. B., *J. Physiol.,* 1920, liv, 1.

[1] Markowitz, J., and Campbell, Walter R., *Am. J. Physiol.,* 1927, lxxxi, 101.

testines by way of the splanchnic and that spinal anesthesia produced a splanchnic block, we decided that spinal anesthesia was dangerous and unnecessary, as the same results could be produced by splanchnic anesthesia by the Kappis technic. Twenty-four dogs were used in this experiment to see if peristalsis could be reestablished in experimentally produced ileus.

Upon opening the abdomen of the dog and exposing the intestine there results a physiological ileus. The number of dogs used in this experiment was 14. This type of ileus was easily overcome by splanchnic block with 1% novocain by the Kappis method, producing active peristalsis in the quiescent intestine. The peristaltic waves, after splanchnic anesthesia, were recorded by kymographic tracings.

The next experiment was the production of a chemical ileus and the reestablishment of peristalsis in this chemically produced ileus. Five dogs were used in this experiment. The ileus was produced by injecting into the abdominal cavity, through the abdominal wall, iodine and potassium iodide solution. Observations were then made fluoroscopically and radiographically by contrast meal introduced into the gastro-intestinal tract. After the development of the ileus, splanchnic anesthesia was produced. Fluoroscopical and radiographical observations demonstrated the return of peristalsis in the ileus, which readily returned to the normal condition, showing that a chemical ileus could easily be overcome and peristalsis reestablished by splanchnic anesthesia.

The next experiment was to produce the mechanical ileus by obstruction of the small bowel and the use of splanchnic anesthesia to overcome this type of ileus. Six dogs were used in this experiment —3 cases of high jejunal obstruction and 3 cases of terminal ileum obstruction. The obstruction was produced under aseptic technic by tying a piece of tape around the bowel until the lumen was obliterated. The high obstruction was allowed to progress for 48 hours. Observations were then made. The terminal ileum obstruction was allowed to progress for 6 days and observations were made. At this period the abdomen was opened and there was found a marked paralytic ileus with distention. As in the physiological ileus, a rubber balloon was introduced into the intestine, in order that peristaltic waves could be obtained by kymographic tracings. In the high and low obstructed cases splanchnic anesthesia was produced and marked peristalsis occurred in both types of obstruction.

Evidence is therefore submitted that physiological, chemical, and mechanical ileus may be satisfactorily treated in experimental animals by blocking the splanchnic nerves with novocain anesthesia according to the Kappis technic.

3936

Effects of In Vivo Prepared Toxic Products of B. Coli Upon the Guinea Pig.*

W. H. HARRIS AND O. M. LARIMORE.

From the Department of Pathology, Tulane University.

In a previous communication,[1] one of us (Harris) reported the production of experimental typhoid fever in the guinea pig by employing certain toxic substances of *B. typhosus*. These toxic materials were obtained by producing primarily a peritonitis by the injection of suspensions of *B. typhosus*. The exudate present in the peritoneal cavity was filtered through a Berkfeld letter N filter and the filtrate used for injections.

The object of the present work was to determine if products of the colon bacillus obtained in a similar manner as employed with *B. typhosus*, would produce the same results as manifested in the typhoid experiments. Cultures of *B. coli communior* were injected into the peritoneal cavity of guinea pigs. Fifteen pigs were employed. The exudate from the resultant peritonitis was diluted and filtered through a letter N Berkfeld filter, and the filtrate injected subcutaneously into a series of guinea pigs. A slight febrile rise was noted following the injections and the leucocytic count was slightly lowered. The animals received several injections and eventually died after 4 to 6 weeks. The post mortem examination of these animals showed but little changes macroscopically excepting in the lungs and kidneys. In the lungs, areas of marked congestion were seen and in the kidneys intense redness and swelling were noted. The spleen and other lymphoid structures of the peritoneal cavity demonstrated but little change. The lymphatic glands were in certain instances slightly enlarged. The Peyers patches did not reveal the changes as previously described for the typhoid filtrate.

The microscopic study of the various organs showed chiefly intense congestion of the blood vessels and degenerative changes of the parenchymatous structures. These findings were noted especially in the kidneys and lungs. In the latter organ hemorrhagic extravasations were often present. The lymphoid structures in addition to the congestion showed occasionally a hyperplasia of both the connective tissue and lymphoid elements. The phagocytic cells prevalent in the animals injected with typhoid toxin, were not found in the guinea pigs given the filtrate of the colon material.

* Aided by a grant from the David Trautman Schwartz Research Fund.

[1] Harris, W. H., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 5, 372.

In these experiments the marked leucopenia and febrile reaction observed for the typhoid filtrate did not occur, the reaction being comparatively slight. The lesions as a whole, both gross and microscopic were those of a toxemia but unlike the representative lesions produced by the typhoid toxin.

It would appear, therefore, that the toxic moiety obtained from *B. coli* wherein a peritonitis has been excited, does not demonstrate the effects analogous to human typhoid fever previously noted when the typhoid bacillus had been employed in a similar manner.

3937

Afebrile Post-Scarlatinal Nephritis.*

CHARLES W. DUVAL.

From the Department of Pathology, Tulane University.

In many cases of scarlet fever the symptoms of the infection have passed and recovery has apparently been established, when acute nephritic symptoms without fever appear about the third week. Of these, the signs of glomerular nephritis are the most prominent. It is generally thought that the new symptoms are not those of a secondary infection or late manifestations of scarlatina. In this connection Longcope[1] and his co-workers indicate that the late nephritis following scarlet fever is the result of existing foci of streptococci in the throat and other parts of the upper respiratory tract. They could not obtain evidence that the streptococci caused the glomerular nephritis by actual invasion of the kidney, as cultures of the blood and urine were negative. These authors suggest that toxin from streptococcal foci in the throat, sinuses, etc., is eliminated by the kidney, thus causing the acute glomerular nephritis.

Our experiments with 17 healthy young dogs support Longcope's contention and furthermore prove that the late acute glomerular nephritis (at least in the dog) is not the result of retained viable streptococci in the body but is due solely to the *in vivo* prepared streptococcal lysate (endotoxin) of Duval and Hibbard.[2] In the recovered experimental dogs which had been infected with living cultures of scarlatinal streptococci, a second or a third dose of

* Aided by a grant from the David Trautman Schwartz Research Fund.

1 Longcope, W. T., *et al.*, *J. Clin. Invest.*, 1927, v, 7.

2 Duval, C. W., and Hibbard, R. J., *J. Exp. Med.*, 1927, xlvi, 379.

"lysate" (toxin *in vivo* prepared) produce a severe and often fatal acute hemorrhagic glomerulo nephritis which in every way corresponds to the afebrile post-scarlatinal nephritis of man. These animals tested before administrating the "lysate" showed more or less lytic immunity; that is, a specific streptococcal lysin was demonstrable in the blood serum and the animals could not be infected with the same dosage of living culture that infected them originally.

The deductions drawn from these experiments are that the animals, though lytically immune, were not apparently anti-endotoxically protected against the toxic product of the scarlatinal streptococcus. The toxin was free to act and did so upon the kidney, particularly the glomeruli. Previous experiments by Duval and Hibbard[3] have shown that the hemolytic scarlatinal streptococcus or its endotoxin has a marked predilection for the kidney.

Experimental scarlatinal streptococcal infection in the dog is of infrequent occurrence unless the dose is large (5 cc. emulsion of the entire 36 hour growth from blood agar slant). On the other hand, young dogs are highly susceptible to the *in vivo* prepared and filtered toxin, and not at all affected by the so-called toxic filtrate from cultures. Dogs infected with the living culture or affected by the "lysate" invariably show acute lesions in the kidneys, and death is apparently due to the destructive changes in the glomeruli of these organs.

While Dick and Dick and others secure from scarlet cultures grown in broth a filterable toxin which reacts intradermally in the human non-immune, we have been unable to obtain any toxic effect with this same material employed upon the guinea pig, rabbit, and *macac rhesus* monkey. However, we have obtained striking skin and general toxic reactions in these animals with the filtered *in vivo* prepared product of hemolytic streptococci. Furthermore, we have elicited in the human non-immune the intradermal reaction to scarlatinal lysate which seems to be more specific than the Dick and Dick toxin of culture filtrate. We believe from the character of the reaction in humans, and the same kind of reaction obtained with non-specific protein products, that the Dick and Dick cutaneous test is not specific. In our hands a variety of bacterial proteins produce much the same reaction. It is interesting in this connection that Zlatogoroff and Derkatch[4] state that neither the toxin production nor the Schultz-Charlton test enable them to confirm the conception of the specificity of scarlet fever streptococci.

[3] Duval, C. W., and Hibbard, R. J., *J. Am. Med. Assn.*, 1926, lxxxvii, 898.

[4] Zlatogoroff, S. I., and Derkatch, W. S., *J. Infect. Dis.*, 1928, xlii, 56.

So complex an organ is the kidney that an injury anywhere has far reaching results. If we consider the intricate histology and physiology of the kidney we can better understand the variety of lesions arising from a single injurious agent. In the same organ there occur great destructive changes, side by side with attempts at healing and compensatory hypertrophy. Though the injurious agent be the same, the lesions are of many different kinds, affecting all parts of the kidney parenchyme, but especially the glomeruli in the case of the scarlatinal streptococcus. Experimentally it is seen that the toxin primarily affects the capillary tufts while the viable organisms produce lesions in the interstitial tissues.

The experimental nephritis with scarlet streptococci in the dog is essentially of 2 types, parallel in every way to the 2 types described for man. The *in vivo* prepared toxin produces an acute glomerular lesion, while the living culture produces a type lesion of the stroma that is a lymphocytic infiltration.

The lysate of scarlatinal streptococci causes in the dog extraordinarily complex changes in the glomeruli. The simplest lesion seems to be an intense engorgement of the capillary whorl with the red blood cells becoming densely packed and appearing as though the hemoglobin was completely dissolved out. In many of the tufts the capillary loops become enormously distended with erythrocytic thrombi. Generally this happens only in parts of glomeruli. The occluded capillaries become further distended by masses of eosin staining material which is homogeneous and apparently formed from fused and destroyed red blood cells. These thrombotic capillary loops may become glued to the wall of Bowman's capsule. In older dead loops of capillaries, connective tissue may partially or completely organize them. In other glomeruli where the capillaries are not blocked by hyalin thrombi, their lumenae contain large numbers of lymphocytic or endothelial cells. It is difficult to determine the exact location and character of these cells since many appear to be outside of the capillaries. In no instance are these cells of the neutrophilic variety. We are inclined to regard these abnormal elements as endothelium because of their manner of staining and the character of the nucleus which is definitely vesicular and surrounded with considerable loosely arranged cytoplasm. In other glomeruli there is noted extensive hemorrhage which in some instances can be traced into the tubule. Sometimes the hemorrhage into Bowman's capsular space is so large as to misplace or crowd out the capillary whorl. When blood escapes into the capsular space the red blood cells fuse into a homogeneous pink staining mass and often become attached to the lining of Bowman's space.

In these masses there commonly occurs connective tissue and epithelial cell invasion, producing the socalled "crescent". With fibrous tissue ingrowth and replacement of the hyalinized capillaries the glomeruli become in part or completely obliterated.

The tubular epithelium is remarkably unaffected, at least until quite late in the glomerular process. Later the lining epithelium, especially that of the convoluted portion of the tubule, becomes swollen, granular and filled with fat droplets. Sometimes the epithelium desquamates and the tubules atrophy. All kinds of casts may at times be demonstrated.

A significant feature of the experimentally induced glomerular nephritis in the dog is the absence of any acute inflammatory products about the involved tufts and tubules. However, in dogs that have received repeated injections of streptococcal lysate, and the urine examination showed previously the existence of acute glomerular nephritis, there was seen upon microscopic study of the kidneys a well advanced sub-acute and chronic interstitial change. In these kidneys there was noted fibrous tissue increase in the areas where the greatest damage had occurred to the glomeruli. In such connective tissue areas many of the tubules were atrophic and the glomeruli destroyed. The less affected glomeruli were actually larger than normal, and certain tubules appeared large and others extremely small.

The experimental work carried out upon dogs with both the living culture of streptococcus scarlatinae and its *in vivo* prepared toxin induces an acute glomerular nephritis. The kidney lesion occurs during the infection and may also be induced in the recovered animal several weeks later, by injections of the *in vivo* prepared lysate.

Aside from the question whether the hemolytic streptococcus of Dick and Dick is the primary excitant of scarlet fever, there is abundant experimental proof that the streptococcus is responsible for the acute glomerular nephritis during the infection and the so-called afebrile nephritic period which occurs weeks later. Longcope's observations upon recovered cases of human scarlet fever certainly indicate that though they have recovered from the infection, they may maintain foci of hemolytic streptococci in parts of the upper respiratory tract, and that these foci may be responsible for post-scarlatinal nephritis of man.

It would appear from the results of our experiments that scarlatinal infection in the dog establishes a lytic rather than an antitoxic immunity. If this is true for the human infection it is logical to suppose that the persistence of hemolytic streptococci upon the mucous membrane of the upper respiratory tract, middle ears, sinuses.

etc., would be the source of an intermittent supply of toxin to the system; and though the lytic action of an already existing immunity render the absorbed streptococcal product more effective for the kidney where in greater part the toxin is eliminated.

A comparative study of the experimentally induced scarlatinal nephritis in the dog with the filtered *in vivo* prepared toxin shows a complete analogy with the afebrile post-scarlatinal nephritis of man.

3938

Serological Evidence of Identity of Duval and Hibbard Measles Coccus with that Isolated by Tunnicliff.

ROY F. FEEMSTER. (Introduced by C. W. Duval.)

From the Department of Bacteriology and Pathology of Tulane University.

In a recent paper Tunnicliff[1] has noted the close immunological relationship between the cocci isolated by her from patients with measles and those isolated by other workers, as evidenced by the phagocytic index. In the study she used 2 strains isolated in this laboratory by Duval and Hibbard[2] from the blood stream of human cases of measles during the height of the eruption; the blood in each case was filtered through a Berkfeld N filter before special media was inoculated, a coccus being obtained from every case cultured.

Similar evidence of this immunological relationship has likewise been demonstrated in this laboratory, based upon agglutination reactions. Three rabbits were immunized by 12 intravenous injections over a period of 70 days—one with a coccus sent us by Tunnicliff, another with a measles coccus isolated by Duval and Hibbard, and a third with a stock strain of *Streptococcus viridans*. The first 4 injections were killed cultures, the last 8 were living cultures.

As shown by the accompanying tables, the sera of the rabbits immunized against the measles cocci each agglutinated almost equally and to high dilutions both strains of the measles coccus, while the *Streptococcus viridans* was only slightly agglutinated by these sera. On the other hand, the serum of the rabbit immunized against the *Streptococcus viridans* very markedly agglutinated the homologous organism, whereas the measles cocci were practically not affected.

1 Tunnicliff, Ruth, *J. Infect. Dis.*, 1927, xli, 267.

2 Duval, C. W., and Hibbard, R. J., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 519.

An unexpected result of these agglutination reactions is the fact that the sera of the measles rabbits agglutinated the *Streptococcus viridans* much more markedly than the serum of the rabbit immunized against *Streptococcus viridans* agglutinated the measles cocci. We have not yet been able to account for this experimentally, but we conjecture that it is because rabbits carry streptococci immunologically related to the viridans, but none related to the measles coccus.

TABLE I.

Agglutination with serum of rabbit immunized against Streptococcus viridans.

	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	Control
Strep. vir.	++++	++++	++++	++++	++++	++++	++++	+++	—
Tunnicliff coccus	—	—	—	—	—	—	—	—	—
Duval & Hibbard coccus	+	—	—	—	—	—	—	—	—

TABLE II.

Agglutination with serum of rabbit immunized against Tunnicliff measles coccus.

	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	Control
Strep. vir.	+++	+++	++	+	?	—	—	—	—
Tunnicliff coccus	++++	++++	++++	+++	+++	++	+	?	—
Duval & Hibbard coccus	++++	++++	++++	++++	+++	+++	++	?	—

TABLE III.

Agglutination with serum of rabbit immunized against Duval and Hibbard measles coccus.

	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	Control
Strep. vir.	++	+	?	—	—	—	—	—	—
Tunnicliff coccus	++++	++++	++++	++++	+++	++	+	?	—
Duval & Hibbard coccus	++++	++++	++++	++++	++++	+++	++	+	—

3939

A Micrococcus Causing a Condition Clinically Very Similar to a Mycosis.

ALDO CASTELLANI.

From the Department of Tropical Medicine, Tulane University of Louisiana.

Morphological and Staining Characters. This coccus for which I have suggested the name *M. myceticus* at times morphologically resembles the gonococcus, at times an ordinary streptococcus. Certain strains when recently isolated are practically gram negative, but after subculturing they usually become gram positive.

Cultivation and Isolation from the Lesions. Absence of growth on ordinary agar when inoculated direct from the lesions. The isolation of the organism is rather difficult, as the organism does not grow at first on ordinary agar and various sugar agars; agar tubes inoculated with pus direct from the lesions do not show any growth; it grows on certain special media, the best apparently being creatinin and uric acid agar, but even on these special media the growth is very scanty at first. Later it can be trained to grow on practically all ordinary media, the best medium being Löffler's serum, but the growth is always scanty.

Creatinin Agar. On neutral creatinin agar the growth is fairly visible, though scanty; it somewhat resembles the delicate growth of certain streptococci on ordinary agar.

Uric Acid Agar. The organism grows on this medium, but more scantily than on creatinin agar.

Growth on Ordinary Media. While apparently the organism does not grow on ordinary media (agar and various sugar agars) when these are inoculated direct with material from the lesions, it grows on such media, though scantily, when transplanted from creatinin and uric acid agar—and it can then be made to grow indefinitely provided subculturing is carried out every 3 days; certain strains may die suddenly.

Glucose Agar. On acid glucose-agar, as a rule, there is no growth; on neutral 4% glucose agar a scanty growth of the organism may take place, but it is usually extremely poor.

Serum. A delicate growth; there is no liquefaction of the medium. After several transplantations the growth is quite good.

Gelatine. No liquefaction.

Biochemical Reactions. When the organism has been trained to grow on ordinary media its biochemical properties can be investigated in the usual way.

Pathological Conditions from which the Organism has been Isolated. I have found the organism for some years in certain cases of chronic condition clinically resembling a streptothricosis, the skin being thickened and showing several nodules and also sinuses. In the open lesions staphylococci and many other organisms are found. In the lesions which have not opened only *Micrococcus mycetìcus* is found.

Pathogenicity of Micrococcus myceticus. In a volunteer the subcutaneous injection in the leg of one-third cc. saline emulsion of the strain of *Micrococcus myceticus* isolated from the patient produced within a few hours a rather painful round red patch the size of a 5 cent piece, with fever, maximum 102° F., which lasted for 24 hours. The patch slowly increased in size, becoming a dark red color until it became more like a rather flat nodule, the center of which slowly softened and spontaneously opened 4 weeks after the injection, a small sinus formed which took 2 months to heal. The subcutaneous injection in rabbits in the abdominal region slowly produces a localized nodule which later on—2 to 4 weeks after the inoculation—softens and becomes open with discharge of pus.

This coccus differs from the usual streptococci in some cultural characteristics (grows at first only on certain special media, etc.) and in the peculiar lesions it produces in man closely resembling mycotic lesions.

I shall be pleased to supply workers interested in the subject with cultures of the micrococcus I have briefly described.

3940

Some New Micrococci: M. Levulosinertis, M. Viscidus, M. Enteroideus, M. Afermentans.

ALDO CASTELLANI.

From the Department of Tropical Medicine, Tulane University of Louisiana.

Micrococcus levulosinertis Castellani. Isolated by me a few weeks ago from a case of stomititis. Pathogenicity not proved. This coccus is interesting as it ferments glucose (acidity only), but not levulose. As a rule when an organism ferments glucose it will always ferment also levulose. In fact this is the first bacterial organism I have come across that does not do so. Litmus-milk is decolorized but the reaction is alkaline. The principal characters of the organism are collected in Table I.

TABLE I.

Action of micrococcus levulosinertis on Fehling reducing sugars.

	Glucose	Levulose	Maltose	Galactose	Lactose	Pentose Arabinose	Xylose	Rhamnose
Micrococcus levulosinertis	A	—	—	A	—	A	A	0

Botanical Position. The fact that the organism ferments glucose but not levulose differentiates this coccus from any other bacterium I know of.

Use of M. levulosinertis in the Identification of Levulose. It is sufficient here to state that if a Fehling reducing sugar is not fermented by *M. levulosinertis* but is fermented by *Bacillus morgani* or *B. canalensis* it must be in all probability levulose.

B. canalensis (or *B. morgani*) + *M. levulosinertis* — } = levulose

Micrococcus viscidus Castellani. Isolated from a case of recurrent severe inflammation of the upper lip. It is gram positive, grows well on all ordinary media, especially well on casein agar. Does not liquefy either serum or gelatine (period of observation, 2 weeks). Its principal characteristic is the following: on agar the growth is viscid and firmly adherent to the medium; if from agar it is transplanted on glucose agar the growth becomes softer and is not in the least adherent to the medium; on transplanting it from glucose on to ordinary agar again the growth becomes again viscid and very adherent to the medium.

Micrococcus enteroideus Castellani. This organism has been isolated by me from stools. The cocci are often arranged in chains and may be elongated, in this way the organism taking at times the appearance of a strepto-bacillus rather than a streptococcus. The organism is usually gram-positive, but not rarely many individual organisms remain decolorized. The growth on agar is very delicate. It does not liquefy either serum or gelatine. The organism is serologically different from any strain of intestinal streptococci or enterococci I have experimented with, and temporarily at least might be considered a new species.

Micrococcus afermentans Castellani. This coccus was isolated from an ulcerative lesion of the skin. Is gram-negative, grows well on ordinary agar, does not liquefy serum. It is characterized by not fermenting any sugar.

I shall be pleased to supply workers interested in the subject with cultures of the cocci I have briefly described in this paper.

3941

Detection of Lactosuria by a Simplified Bacterial Method.

ALDO CASTELLANI.

From the Department of Tropical Medicine, Tulane University of Louisiana.

The following is a simplified technic of the so-called Castellani-Taylor mycological method,[1] which I have found most useful in practice. The urine is collected in a clean bottle or other clean vessel, if possible sterile, but this is not essential; if it cannot be examined at once, it can be kept for some hours or even a day or two in the ice-box.

A portion of the urine is boiled for 2 minutes; I have found that boiling for 2 minutes does not apparently alter the chemical structure of lactose in the urine; the boiled urine is then distributed into 2 fermentation tubes, 1 and 2. As soon as the urine has cooled down, No. 1 tube is inoculated with *B. coli* from an agar culture: 2 or 3 large loopfuls; No. 2 tube is inoculated with *B. paratyphosus* B in the same way. The 2 tubes are placed in the incubator at 35-37° for 12-24 hours. The results are then read. If tube No. 1 *(B. coli)* shows presence of gas and tube No. 2 *(B. paratyphosus* B) does not show presence of gas, the inference is that the urine contained lactose.

The explanation is the following: with regard to Fehling reducing sugars which may be found in the urine, *viz.*, glucose, levulose, maltose, galactose, pentose—*B. coli* and *B. paratyphosus* B have the same fermentative reactions except on lactose which is fermented by *B. coli* and not by *B. paratyphosus* B. If a Fehling reducing sugar, therefore, is fermented by *B. coli* and not by *B. paratyphosus* B the inference is that it is lactose. The test may be employed also as a roughly quantitative test using graduated fermentation tubes.

If both tubes show fermentation there are several possibilities: as a rule it is glucose, but it may be levulose or galactose or maltose or pentose or a mixture of them or it may be one or several of these sugars + lactose. It will suffice to say here that if it is suspected that lactose is present in addition to some other Fehling reducing sugar the urine is exhausted with *B. paratyphosus* B and after filtration through a Chamberlain filter is inoculated with *B. coli;* if fermentation takes place the inference is that lactose was present in addition to some other Fehling reducing sugar.

During January and February of this year, thanks to the kind-

[1] Castellani, Aldo, and Taylor, F. E., *Brit. Med. J.*, 1917, ii, 855.

ness of Professors C. Jeff Miller and E. L. King and Doctors Thomas B. Sellers and C. D. Dawson, I have been able to investigate 12 cases of nursing women presenting Fehling reducing urine. In most cases the reaction was slight and disappeared after a few days. The bacterial method showed that in every case the Fehling reducing substance was lactose; no glucose or any other sugar apart from lactose was present.

I shall be pleased to supply workers interested in the subject with cultures of the strains of *B. coli* and *B. paratyphosus* B I use.

3942

Some New Bacilli: Bacillus Metaflavus, Bacillus Flavoides, Bacillus Multiformis, Bacillus Canalensis, Bacillus Pomodoriferus.

ALDO CASTELLANI.

From the Department of Tropical Medicine, Tulane University of Louisiana.

Bacillus metaflavus Castellani. This bacillus I have grown several times from scrapings of the skin of the axillary and pubic regions. It is a most peculiar organism, and it is very doubtful whether it is a bacillus at all; it is gram-negative.

In preparations from agar cultures the organism appears as a long straight, or more often curved, large-sized bacillus; in preparations made from glucose agar cultures 48 hours old globular bodies, cryptococcus-like, 3-7 microns in diameter, are seen in addition to short, thick, bacillary forms. The growth on agar and glucose agar is of a bright yellow color, the surface of the growth may be smooth and shiny or may be crinkled. In stained preparations the organism at times appears as a diplo-bacillus with a large capsule.

Bacillus flavoides Castellani. This bacillus has been isolated by me from scrapings of the skin of the axillary and pubic regions. It is Gram-negative. It grows well on all ordinary media, it does not produce gas in any sugar. The growth on glucose agar is yellow, but after repeated transplanting the power of producing pigment is almost lost. It is easily differentiated from *B. metaflavus* as in preparations from glucose agar cultures no cryptococcus-like bodies are found in addition of the bacillary forms.

Bacillus(?) multiformis Castellani. This is a most peculiar organism isolated from scrapings of human skin. It is gram negative, not acid fast, is easily stained by the ordinary anyline dyes. The

organism may take the most different morphological appearances, especially in hanging-drop cultures; in the same preparation globular cryptococcus-like bodies (2-5-7 microns in diameter) may be present, in addition to thick bacillary forms and most peculiar elongated sinuous forms. The classification is difficult and the possibility of its being a true cryptococcus cannot be discarded, although against it there is the fact that the organism is gram negative and the dimensions are smaller than in most cryptococci and moreover many bacillus-like forms are present. For convenience's sake I will consider it a bacillus and call it *B. multiformis*.

Bacillus canalensis Castellani. A motile gram negative asporigenous bacillus isolated from human stools. Some strains seem to be non-motile. It grows well on ordinary media. It does not liquefy gelatine, it does not liquefy serum and does not clot milk.

With regard to the usual carbohydrates used in laboratories it ferments with production of gas only glucose and levulose. It differs from *B. morgani* as in contrast to the latter it does not ferment galactose.

Bacillus pomodoriferus Castellani. This somewhat peculiar bacillus was isolated from the urine of a case of cystitis and also from a stool. Its pathogenicity has not been proved. It is non-motile, gram negative, asporigenous, does not produce either acidity or gas in any sugar; it slowly liquefies gelatine and serum. Its principal characteristic is the production in young cultures, especially in serum cultures, of a peculiar rather pleasant penetrating odor resembling the smell of apples. In old cultures the odor disappears or becomes disagreeable.

I shall be pleased to supply workers interested in the subject with cultures of the microorganisms I have briefly described in this paper.

Peking Branch.

Peking Union Medical College, February 9, 1928.

3943

Body Metabolism and the Induction of Blood Clotting.

H. NECHELES, C. A. MILLS AND MAO-KENG CHU.

From the Departments of Physiology and Medicine, Peking Union Medical College.

It was shown in a previous communication[1] that protein ingestion causes a decrease of the clotting time in man and dogs, while fat and carbohydrate have little influence. Further work[2] showed that these changes in clotting time are closely accompanied by changes in metabolism—as the metabolic rate increases the clotting time shortens. Ingestion of food with little or no specific dynamic action has practically no effect on the clotting time. Amino-acids have the same effect as proteins.

The question arose as to whether every increase in metabolic rate, no matter how produced, would shorten the clotting time; also whether the clotting time could be shortened without an accompanying increase in metabolism; and finally what caused the decreased clotting time. We suspected that the platelets were responsible, so their behavior was studied.

The experiments were carried out on one of us (C. A. M.) and on dogs. One dog was tracheotomized, the wound permitted to heal thoroughly and a Trendeenburg tampon-cannula used at the time of metabolism study. A Benedict-Knipping[3] closed circuit metabolism apparatus was employed for the determinations.

Blood was drawn from a vein with a paraffined needle and syringe and placed in a paraffined tube at 25° C. Every 2 minutes a sample of the blood was removed by a paraffined pipette and a smear quickly made on a glass slide. This smear was stained with

[1] Mills, C. A., and Necheles, H., *Chinese J. Physiol.*, 1928, ii, 19.

[2] Necheles, H., and Mills, C. A., *Chinese J. Physiol.*, 1928, ii, 25.

[3] Knipping, H. W., *Z. Physiol. Chem.*, 1925, cxlv, 154.

Wright's stain and the speed of platelet clumping and disintegration observed.

The metabolic rate of man and dog was increased by feeding protein. The specific dynamic action was accompanied by a shortened clotting time, while complete clumping of the platelets occurred about the same time as the first appearance of clot in the tube. Glycocoll feeding gave the same changes in the dog as did protein. Fat and carbohydrate feeding with the dog, and carbohydrate and fat-carbohydrate meals with man, gave insignificant changes in the metabolism, clotting time and platelet clumping. The metabolic rate in the dog was next raised by feeding dried thyroid extract. When the metabolism was thus raised to +30%, no change could be detected in the blood clotting or platelet clumping. The metabolic rate was next markedly increased in both man and dog by vigorous exercise, and this was always found to cause a marked quickening of both clotting and platelet clumping. Injections of adrenalin into dogs gave results similar to those following vigorous exercise.

To shorten the clotting time without affecting the metabolic rate, tissue fibrinogen was injected into the dog. Two hours later the clotting time had been reduced by 80% and the platelet clumping correspondingly quickened. There was found no change in the metabolic rate, so this coagulent affects the coagulability by a mechanism independent of basal metabolism.

In vitro experiments with amino acids and different protein metabolites failed to show an accelerating effect of any of them on blood clotting, when used in physiological concentrations. Adrenalin effect *in vitro* is now being tested.

We have here demonstrated that not every increase in metabolic rate is accompanied by a quickened clotting of the blood and platelet clumping, and also that not every decrease in the clotting time is accompanied by an increased metabolic rate.

Metabolic changes which we commonly think to be associated with increased adrenal secretion always seem to be accompanied by quickened clotting.

Apparently the platelet clumping and disintegration are responsible for the time of onset of the clotting process. We are now studying the influence of adrenalin on platelets and platelet-free plasma *in vitro*.

3944

Selection of the Number of Tailrings in the Albino Mus Musculus.

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From the Department of Anatomy, Peking Union Medical College.

The tail of a mouse is characterized by scales which are arranged in more or less regular rings. Taxonomists long ago recognized the systematic value of the average number of tailrings. For *Mus musculus* this is given at about 180.

In 400 albino mice obtained from the animal house of the Peking Union Medical College—all of them descendants of 32 animals imported from the Rockefeller Institute, Princeton, N. J., by Dr. C. TenBroeck in 1924—the number of tailrings, counted after the death of the animal, varied from 142 to 220, with an average of 189.38 ± 0.62. (The standard deviation is ± 12.57.) On account of the irregularity of some rings, the number of tailrings of a given mouse cannot be stated with absolute accuracy; it may be 2 more or less than the number actually counted. The correlation of the number of tailrings with the length of the tail is low. (The coefficient of correlation is 0.27 ± 0.05.) In general, therefore, fewer rings mean broader rings.

Selection of the number of tailrings in these mice was tried simultaneously in the direction of high and of low number and all animals were kept in the same room and treated in the same way. This prevents crediting any changes in the number of tailrings to changes in the environment.

Selection was based on the number of tailrings counted in the living mouse. For the low number of tailrings (series L T M) 4 mice were chosen which appeared after death to have 152, 154, 178, and 196 tailrings. Of course, the selection of the last one for this group was a mistake. After 7 months these 4 mice had altogether 65 descendants. In 58 of them the tailrings were counted after death. Their number varied between 149 and 212 with an average of 170.15 ± 1.77. (Standard deviation ± 13.49.) The result of a second selection is not yet accurately known as most of the animals concerned are still living, but the number of tailrings seems to have reached an average of about 148 and extremes of 134 and 156.

For the selection of a high number of tailrings (series H T M) 9 mice were chosen which after death appeared to have 194, 200, 201, 205, 208, 209, 212, 222, and 225 tailrings. (Average 208.) These 9 mice had after 7 months a total of 63 descendants. In 50

of them the tailrings were counted after death. Their number varied between 172 and 228 with an average of 207.38±1.53. (Standard deviation ±10.83.)

From the 63 descendants of the first group 10 animals were again selected. After death 8 of them appeared to have 211 to 227 tailrings, with an average of 217. Within 3 months this second group of 10 selected animals had 53 descendants. In 47 of them the tailrings were counted after death. The number varied between 187 and 220 with an average of 204.06±1.23. (Standard deviation ±8.48.)

This second selection had no greater success than the first selection in the H T M series. This proves that a fairly pure race with a higher number of tailrings had been isolated from the original stock by the first selection. The original colony also contains a race with a low number of tailrings, but this has not yet been successfully isolated. How many other races with intermediate numbers of tailrings may be selected from the original colony remains uncertain.

The study of these races of *Mus musculus* with widely differing numbers of tailrings will be continued, but the results of the experiments already appear to justify the opinion that in the phaenotype of the mouse a new detail has been found which may serve as basis for genetical analysis.

3945

Some New Alkaloids from Chinese Corydalis Ambigua, Cham. et. Sch. (Yen-Hu-So).

TSAN-QUO CHOU. (Introduced by B. E. Read.)

From the Department of Pharmacology, Peking Union Medical College.

Corydaline, the principal alkaloid of Corydalis roots, was discovered by Wackenroder[1] in the tubers of *Corydalis tuberosa*. It was subsequently examined by several investigators, but not in a pure state until Dobbie and Lauder[2] analyzed the pure alkaloid and its salts and assigned to it the formula $C_{22}H_{28}O_4N$. Freund and Josephy[3] found that the alkaloid was better represented by the formula $C_{22}H_{27}O_4N$, which is now generally adopted. A dozen other

[1] Wackenroder, cited by *Kastner's Archiv.*, 1826, viii, 423.

[2] Dobbie, J. J., and Lauder, A., *J. Chem. Soc.*, 1892, lxi, 244.

[3] Freund, M., and Josephy, W., *Annalen*, 1893, cclxxvii, 1.

alkaloids were successively isolated from *Corydalis cava* and other species of Corydalis roots by Dobbie and Lauder,[4] Freund and Josephy,[5] Merck,[6] Gadamer,[7] Spath,[8] Heyl,[9] Makoshi,[10] Asahina,[11] and others, to most of which molecular and constitutional formulae have now been assigned. The Chinese Corydalis tubers, Yen-hu-so, are, according to Matsumura[12] and Stuart,[13] *Corydalis ambigua, Cham. et Sch.* They are small, firm, brownish-yellow pellets, with a depression on one of the surfaces. To the drug itself, in China, are ascribed tonic diuretic, emmenagogue, deobstruant, astringent, alterative and sedative properties. Its chemical investigation was first carried out by Makoshi,[10] who isolated from it corydaline, dehydro corydaline, Corbulbine, protopine, and 2 other alkaloids, $C_{20}H_{17}O_4N$, a quaernary base and a substance m. p. 197-199°, resembling, but not identical with bulbocapnine. With the object of obtaining some bulbocapnine for certain medical requirements, the writer investigated chemically the Chinese drug Yen-hu-so again and found that it contains more alkaloids than had hitherto been isolated. The basic products, obtained from 16 kg. of crude drug, were divided into 4 fractions A, B, C, D. The fraction B, which is non-phenolic and consists of the largest part, weighing about 20 gm., was first studied, from which 5 alkaloids have been isolated, purified, analyzed and some of their salts prepared. One of these is identical with Corydaline in all respects and the other 4 are new. They are named provisionally *Corydalis* A, *Corydalis* B, etc., until further confirmation.

1. *Corydalis* A. (Corydaline) $C_{22}H_{27}O_4N$ m.p. 135° $[\alpha]$ 25/D $= +295°$. Crystallized from alcohol in six-sided prisms. Ethyl sulphate, m.p. 162°; hydrochloride, m.p. 214°; nitrate, m. p. 197°; platinit chloride, m.p. 227°; methiodide, m.p. 228°. It is identical with Corydaline in all respects.

2. *Corydalis* B. (new alkaloid) $C_{20}H_{23}O_4N$ m.p. 148-149° $[\alpha]$ $D = 0°$. Recrystallized from alcohol in plates, hydrochloride, needles, m.p. about 218°; and oxalate, prisms, m.p. 208°. This

[4] Dobbie, J. J., and Lauder, A., *J. Chem. Soc.*, 1895, lxvii, 25.

[5] Freund, M., and Josephy, W., *Ber.*, 1892, xxv, 2411.

[6] Merck, E., *Arch. Pharm.*, 1893, ccxxxi, 131.

[7] Gadamer, J., Ziegenbien, H., and Wagner, H., *Arch. Pharm.*, 1902, ccxl, 19; *ibid.*, 1911, ccxlix, 30.

[8] Spath, E., Mosettig, E., and Trothandt, O., *Ber.* 1923, lvi, 875.

[9] Heyl, G., *Apoth. Zeit.*, 1910, No. 17 reprint.

[10] Makoshi, K., *Arch. Pharm.*, 1908, ccxlvi, 381.

[11] Asahina and Motigase, *J. Pharm. Soc. Japan*, 1920, 463, 766.

[12] Matsumura, J., "Chinese Names of Plants," 1915.

[13] Stuart, G. A., "Chinese Materia Medica," 1911.

alkaloid has the same m.p. as that obtained by Heyl[9] from the roots of *Corydalis aurea,* but differs from it in its color reactions toward HNO_3 and Erdmann's reagent. Heyl did not give its specific rotation and molecular formula.

3. *Corydalis* C. (new alkaloid or Protopine?) $C_{20}H_{19}O_5N$. m.p. 201°; $[\alpha]/_D = 0°$. Recrystallized from a mixture of chloroform and alcohol in nodular mass or prisms, hydrochloride, prisms, m.p. 248°; acid oxalate, prisms, m.p. 237°; aurochloride m.p. 195°; hydrobromide, prisms, m.p. 250°. This alkaloid is similar to Protopine in its molecular formula and some color reactions towards H_2SO_4. Froedes and Erdmann's reagents, but its m.p. remains constant at 201° after repeated crystallization from a mixture of alcohol and chloroform instead of 207° m.p. of Protopine.[14]

4. *Corydalis* D. (new alkaloid) $C_{19}H_{16}O_4N$ or $C_{19}H_{17}O_4N$. m.p. 204°. $[\alpha]^{25}/_D = -295°$. Recrystallized from a mixture of alcohol and chloroform in prisms. Hydrochloride, fine needles m.p. about 250°; hydrobromide, crystalline powder, m.p. about 260°. This is the first alkaloid of Corydalis series having a very high leavorotatory power.

5. *Corydalis* E. (new alkaloid). Recrystallized from chloroform and alcohol in long needles m.p. 219°, hydrochloride, m.p. 246°. The small amount of this alkaloid in hand does not permit the writer to determine its specific rotation and molecular formula.

Further study of these alkaloids as well as the isolation of other alkaloids from the other 3 fractions A, C, D, is in active progress. The physiological properties of *Corydalis* B and *Corydalis* C have been studied respectively by Drs. H. P. Chu and C. Pak.[15] The results are interesting and promising medically.

The following is a summary of their results:
Corydalis B (by H. P. Chu).

1. It produces general narcosis when given subcutaneously into a rat weighing 81 gm. With 10 mg., sleep sets in promptly and lasts for about 2 hours. It is not preceded by any symptoms of excitement.

2. It promotes local anesthesia when applied to the scarified areas on the extensor surface of the fore arm. The sensibility to pain becomes completely abolished within 5 minutes and the effect persists for about an hour.

3. In cats, with 5-10 mg., it produces a slight rise of blood pressure, which is accompanied by a decrease in the rate and an in-

[14] Danckwortt, P. W., *Arch. Pharm.*, 1912, ccl, 590.
[15] Private communications.

crease in the height of contractions of the heart. The cardiac effects are also observed directly on the isolated strips of the heart. In an excised right sinus auricle of the cat 0.002% to 0.01% produces a slowing of the rate and increases excursions.

Corydalis C (by C. Pak).

Non-toxic doses of *Corydalis* C produce a depression of the central nervous system or slight convulsion in frogs, and violent chronic convulsions in rats and rabbits, which are chiefly confined to the head, neck and dorsal muscles. The poisoned animals recover rapidly. The convulsions are independent of reflux stimulation. In decerebrated rat, *Corydalis* C causes no convulsions and its action is probably, therefore, located in the cerebrum. The blood pressure shows in rabbit a primary fall and a secondary rise. The respiration is accelerated.

New York Meeting.

College of the City of New York, April 18, 1928.

3946

Method for Electrometric Titration of Organic Acids of the Blood.

WILLIAM A. PERLZWEIG AND GEORGES DELRUE.*

From the Chemical Division of the Medical Clinic of the Johns Hopkins Hospital and University.

Repeated attempts were made to apply Van Slyke and Palmer's[1] titrimetric method for the estimation of the organic acids of the blood plasma. These attempts were unsuccessful chiefly because with the small concentrations involved, no satisfactory end-point in the acid range could be obtained with the available indicators (thymol blue, bromphenol blue, Tropaeolin 00). In order to overcome this difficulty and also in the hope of gaining some information concerning the nature of the organic acids from the titration curves, the following method for the electrometric titration of these acids in the blood was developed.

The proteins are removed by means of ultrafiltration through collodion membranes or by precipitation with metaphosphoric acid; the carbonates, phosphates, oxalates and sugar are removed by addition of $CuSO_4$ and solid $Ca(OH)_2$. The filtrate may at this point be evaporated on the water bath to a small volume. More calcium hydroxide, sulfate and carbonate as well as basic cupric carbonate usually crystallize out on evaporation. The contents of the evaporating dish are passed through a small filter with suction washing the precipitate with several small portions of water until the volume of filtrate corresponds to 1.5 to 2 times that of the sample of plasma in the filtrate taken for evaporation. An aliquot corresponding to (3 to 5 cc. of plasma) of the alkaline filtrate is then placed in a simple electrode vessel containing a drop of methyl orange solution, and

* Committee for the Relief of Belgium Fellow, 1926-1927.

[1] Van Slyke, D. D., and Palmer, W. W., *J. Biol. Chem.*, 1920, xli, 567.

N HCl is added to a definite acid reaction. A small amount of quinhydrone is then added, the vessel is shaken and placed in a small water bath at 20° and is connected by means of a saturated KCl-agar bridge with a saturated calomel electrode suspended in the same water bath. The other connections are then made with any potentiometer circuit. A stream of nitrogen bubbling through the solution furnishes a convenient method of stirring and of removing CO_2. More *N* HCl is added until an arbitrarily chosen potentiometer reading is reached, corresponding approximately to pH 2.30. The titration is then carried out in the customary way from a microburette with CO_2 free 0.1 *N* NaOH to a sharp drop of the P. D. at or slightly beyond the change of polarity. A control titration is carried out with the same volume of a solution obtained by treating a 0.1 *M* NaCl solution with metaphosphoric acid, $CuSO_4$ and evaporation, etc., exactly in the same manner as and simultaneously with the plasma. The calomel electrode may be conveniently checked with every set of titrations by means of Machaelis' standard acetate (pH 4.62) buffer. The control and the plasma curves are now plotted together. The total organic acid may be read off between the endpoints of the upward inflections of the control and of the plasma curves. The calculation of the pH values from the potentiometer readings may be very simply obtained by reference to the value obtained with the standard buffer whose pH is 4.62,

$$pH = 4.62 - \frac{E_0 - E_1}{\frac{RT}{F}}$$

where E_0 is the observed potential in volts of the standard acetate buffer of pH 4.62 and E_1 is the observed potential in volts of the titrated solution. The RT/F factor (0.000, $1984 \times 273 + t$) is a constant for any given temperature and may be readily obtained from tables; at 20° C. it is 0.0581. The correction for creatine and creatinine as formulated by Van Slyke and Palmer[1] is negligible in the case of blood serum or plasma.

The plotted curves permit one to estimate the total amount (equivalents) of organic acid present. This quantity is represented in the abscissa of the curve between the points of maximum inflection of the control and the blood filtrate solutions. Furthermore, if a relatively smooth curve between the above 2 points is obtained, indicating the presence of 1 organic acid or more than 1 organic acid whose dissociation constants are in close proximity to each other, the dissociation constant (pK) of the 1 acid or the approximate

(composite) pK value of the mixed organic acids can be estimated. This is done in the usual manner by reading off from the curve the pH value at which the organic acid is exactly one half neutralized, corresponding to the equation

$$pH = pK + \log \frac{(salt)}{(acid)}$$

and $pH = pK$ when the (salt)/(acid) ratio becomes equal to 1, *i. e.*, when the acid is exactly one half neutralized.

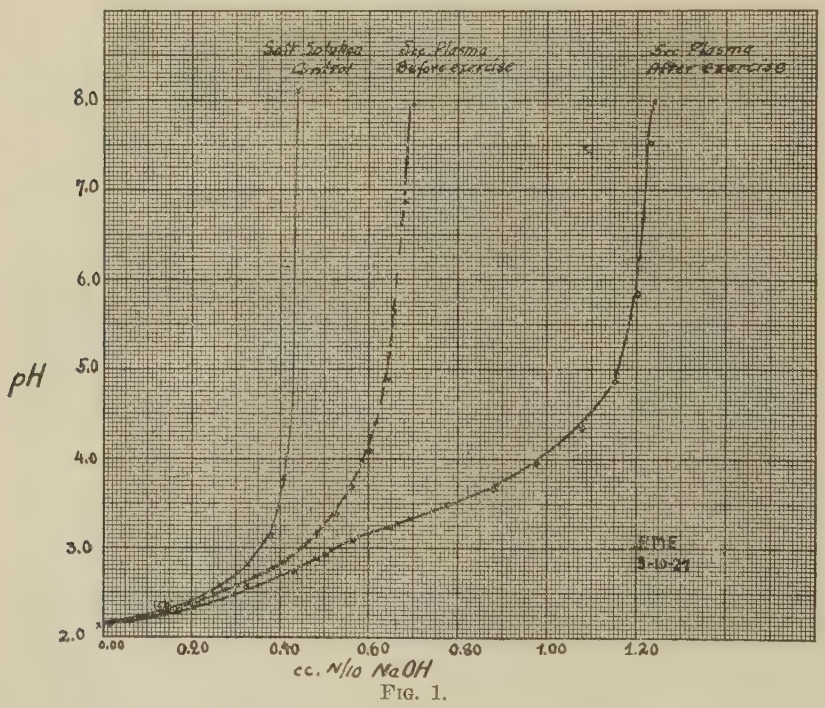

FIG. 1.

In Fig. 1 are shown the titration curves of plasma before and after vigorous exercise.

The concentration range of organic acids in normal blood, serum and plasma has been found to vary between 5 and 8 milliequivalents per liter. It has also been observed that the composite pK value of the organic acids of most of the blood samples studied by this method lies with remarkable regularity between 3.6 and 3.8. The significance of this observation as bearing upon the identity of the organic acids involved as well as variations of the concentration of organic acids in the blood under various conditions is being investigated further.

3947

Rickets in Rats. V.* Comparison of Effects of Irradiated Ergosterol and Cod Liver Oil.

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From the Laboratory of Physiological Chemistry, Yale University.

Hess, in collaboration with Windaus[1] and others, have achieved the cure of rickets by minute amounts of irradiated ergosterol. Most of the studies on the cure of rickets in experimental animals are based upon the X-ray pictures, histological preparations of the bones, and blood serum analyses for calcium and phosphorus. Quantitative data are, however, supplied only by metabolism studies[2, 3, 4] and the analyses of the ash of the dry fat-free bones.[5, 6, 7] By use of technique described elsewhere[8, 9] we desired to compare by the various analytical procedures the cure of standardized ricketic rats by cod liver oil and ergosterol.

Animals fed on Sherman Diet B, when 28 days old were placed on Steenbock and Black's high calcium-low phosphorus diet 2965 plus 10% lard. By analysis the diet contained 1.07% calcium and .178% phosphorus. The ratio of Ca/P was 6.0. They were kept on this regime for 21 days. Then the diet was altered by replacing 2% of the lard by cod liver oil, or irradiated ergosterol in olive oil.† Both were fed separately and not mixed with the diet. The amount of ergosterol represented .01 mg. per rat per day.

The animals were killed after 14 days on the curative diet. The autopsies showed intramuscular hemorrhages in the upper hind ex-

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

1 Hess, A. F., *J. Am. Med. Assn.*, 1927, lxxxix, 337.

2 Hess, A. F., and Sherman, E., *J. Biol. Chem.*, 1927, lxxiii, 145.

3 Boas, M. A., *Biochem. J.*, 1926, xx, 153.

4 Schultzer, P., *Biochem. Z.*, 1927, clxxxviii, 427.

5 Bethke, R. M., Steenbock, H., and Nelson, M. T., *J. Biol. Chem.*, 1923, lviii, 71.

6 Dutcher, R. A., Creighton, M., and Rothrock, H. A., *J. Biol. Chem.*, 1925, lxvi, 401.

7 Chick, H., Korenchevsky, V., and Roscoe, M. H., *Biochem. J.*, 1926, xx, 622.

8 Karelitz, S., and Shohl, A. T., *J. Biol. Chem.*, 1927, lxxiii, 665.

9 Shohl, A. T., and Bennett, H. B., *J. Biol. Chem.*, 1927, lxxiv, 247.

† The irradiated ergosterol was generously supplied us by Dr. Charles N. Frey of the Fleischmann Laboratories, New York City. This was irradiated in ether for 30 minutes at 28 inches with a mercury arc lamp. The ergosterol was taken up in olive oil.

tremities of 2 of the rats which received ergosterol. The rats were studied as to (1) blood serum analyses, (2) histological examination of the bones, (3) bone analyses, and (4) metabolism of calcium and phosphorus.

TABLE I.
The analyses of the blood serum for calcium and phosphorus.

	Calcium mg. %	Phosphorus mg. %
Cod liver oil	13.8	5.5
Irradiated Ergosterol	11.7	5.8
Rickets Control	9.9	3.7

These rats were made ricketic on Steenbock's diet 2965 plus 10% lard for 21 days and then given additions of the curative agent for 14 days.

1. *The blood serum analyses,* as shown in Table I, indicate a marked rise in the serum phosphate above that of the rickets control with both cod liver oil and irradiated ergosterol. The calcium is also definitely raised. This degree of elevation of the serum phosphate is never present as a consequence of this diet unless rickets has been cured.

2. *The histological preparations* show marked unhealed rickets in the rickets control group. The bones of the group receiving cod liver oil and that with ergosterol show substantially the same condition. A narrow metaphysis is present; calcification in the provisional zone is complete and in a straight line; the trabeculae are straightening and osteoid tissue is being resorbed. All these are indications of moderately advanced healing.

TABLE II. *Analysis of femur.*
Values in terms of one femur.

	Rickets Control	Cod Liver Oil	Irradiated Ergosterol
	mg.	mg.	mg.
Wet weight	240.0	280.0	280.0
Dry weight	107.0	136.0	133.0
Fat free	90.0	100.5	110.0
Organic	61.7	56.4	60.5
Ash	28.3	45.0	49.5
Ash in % of fat free	31.6	45.0	45.0
A/R	0.46	0.80	0.82

Analysis of the bones of the rats at the age of 63 days.

3. *The analysis of the bones,* as shown in Table II, indicate marked deficiency in calcification in the rickets control group. Those receiving cod liver oil and ergosterol have definite increase in their ash content. At this age the bones of normals contain about 60% ash, but the normal rate of increase of ash is exceeded by that of the animals which have been cured with cod liver oil or ergosterol.

TABLE III.

Metabolism of Calcium and Phosphorus.

Figures in terms of 1 rat per week.

	Food	Stool	Urine	Spill	Total	Bal.	Food	Stool	Urine	Spill	Total	Bal.	Ca/P in Bal.	Stool	Weight inc.
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.		gm.	gm.
Irradiated Ergosterol															
Period I	506	331	115	—70	376	130	84	58.8	2.5	—8	54	30	4.35	3.58	7.0
Period II	506	360	110	—60	410	96	84	63.	14.1	—8	77	7	13.0	3.93	7.7
Cod L. O.															
Period I	548	254	99	—10	343	205	90	51.4	2.1	—1.3	52	38	5.4	3.64	4.1
Period II	505	295	113	—14	394	211	83	59.5	2.3	—3	59	24	8.8	4.22	7.1

See footnote to Table I.

4. *The Metabolism of Calcium and Phosphorus.* The data given in Table III show that the food intakes, the weight of the feces and the growth of the rats are similar to those on the rickets control diet.[9] The analysis of the food revealed that the phosphorus con-

tent was extremely low compared to that used in other experiments. The phosphorus was .177% instead of .254%. The ratio of Ca/P, which in former experiments was 4.25, in this experiment was 6.0.

The metabolism data demonstrate that the cure of rickets is not necessarily associated with great increase in the phosphorus balance. On this severe diet, negative balances of phosphorus would probably result without the curative agent. In the rickets controls, the calcium is retained in excess of the phosphorus; in the cured animals even larger amounts of calcium were retained. This indicates that healing of the condition causing deficient bone deposition may be effected by much efficient utilization of the phosphorus as well as by increase in the retention.

Summary: Marked cure of rickets in rats is secured in 2 weeks, by cod liver oil and by .01 mg. daily of irradiated ergosterol. This is shown by histological preparations of the bones, the analyses of the blood serum for calcium and phosphorus and the ash analyses of the bone. The metabolism studies demonstrate that the cure is accomplished without great increase in the retention of calcium or phosphorus.

3948

Prediction of the Basal Metabolism of Infants from the Measured Insensible Perspiration.

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From the Department of Pediatrics, Cornell University Medical College, and the New York Nursery and Child's Hospital.

Measurement of the respiratory exchange of infants by present methods is time-consuming and technically difficult. The result is that only 3 or 4 pediatric clinics in America are making any attempt at present to study the subject. This paucity of data is regrettable since a knowledge of the energy metabolism furnishes the scientific basis for the feeding of infants in health and in nutritional disturbances, besides yielding valuable information in many other conditions. For these reasons, the attempt has been made to extend to infants the method proposed by Benedict and Root[1] for predicting the probable metabolism of adults from the insensible perspiration as measured with the aid of a delicate balance. The results thus far obtained with the method are presented in this preliminary report.

[1] Benedict, F. G., and Root, H. F., *Arch. Int. Med.*, 1926, xxxviii, 1.

The method is based on the close relationship which exists in the human organism from early infancy to old age between the production of heat and the loss of heat by way of vaporization of water through the skin and lungs. Du Bois[2] has shown that under standard conditions of clothing, temperature and humidity, healthy adults at rest and in the post-absorptive state lose approximately 25% of their heat in vaporization through the above channels. This relation was maintained in all of the afebrile diseases investigated.

Simultaneous measurements of heat production and water elimination in the respiration chamber at the New York Nursery and Child's Hospital demonstrated that under comparable conditions normal and marasmic infants lose the same proportion of heat in vaporization as adults; namely, 26%, the extreme values being 23 and 30%.[3] The chart shows this relation between heat production, expressed as calories per 24 hours, and insensible perspiration, expressed as gm. per hour. The term insensible perspiration, as commonly used and as recorded in the chart, includes other gaseous emanations than water vapor, the chief component being carbon dioxide. Atmospheric oxygen is absorbed at the same time as carbon dioxide is exhaled. The difference or the weight of the carbon

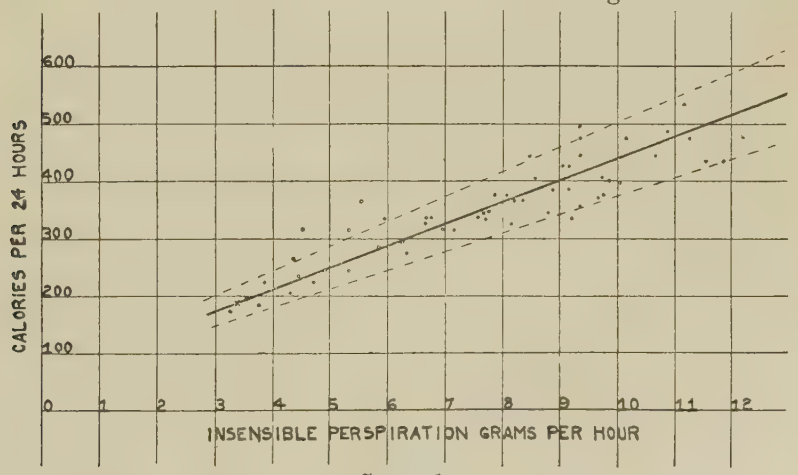

CHART 1.

Relationship in infants between the insensible perspiration per hour and the heat production per 24 hours as simultaneously measured in the respiration chamber. Dots indicate normal infants; circles, marasmic infants, and the cross, a cretin. The straight line curve represents the general trend of the points. The area between the dash lines indicates a deviation of 15% from the straight line curve.

[2] Soderstrom, G. F., and DuBois, E. F., *Arch. Int. Med.*, 1917, xix, 931.

[3] Levine, S. Z., and Wilson, J. R., *Am. J. Dis. Child.*, 1928, xxxv, 54.

together with the water vapor constitutes the total loss of weight or insensible perspiration. The fraction ascribable to carbon is between 10 and 15%, depending on the respiratory quotient.

Although observations are as yet too few to justify mathematical treatment a straight line curve correlating heat production with insensible perspiration has been tentatively drawn, according to the method proposed by Benedict and Root for adults. The scatter of individual points, each of which represents a single observation in the respiration chamber is relatively slight and does not exceed the scatter found on most standard charts of basal metabolism. In all, 58 observations were made on 18 infants. Only 9 of the 58 points deviate from the arbitrary curve, as laid out, by more than 15%. Only 3 of the points exceed a deviation of 20%. When one considers the many errors inherent in the measurement of water output in the respiration chamber, one may reasonably conclude that the discrepancies noted above may be ascribable as much to errors in technic as to intrinsic variations in the water output of individual infants.

The existence in infants of a condition of equilibrium between heat production and insensible perspiration induced us to make an attempt to predict their metabolism from their insensible perspiration measured outside of the respiration chamber. The technic of measurements consisted briefly of weighing infants on a delicate balance having a capacity of 10 kg. with an accuracy of 20 mg. under standard conditions of clothing, temperature and humidity. Observations included one or more half- to one hourly periods and were invariably made during sleep, in the absence of visible perspiration and at least 1 hour after the ingestion of a small meal. The frame and rubber mattress on which the subjects lay were specially constructed to permit the collection of urine and feces without loss. A complete description of the apparatus, details of the method, sources of error and precautions taken to avoid these errors will appear in a later communication.

To date, 9 normal infants, 6 marasmic infants and 1 cretin have been studied in 23 basal observations by the method of weighings. The heat production of 8 of the infants was also measured in the respiration chamber either on the same day or within a day or 2 of the weighings under the same basal conditions. The basal heat production of the remaining infants was estimated from the standard tables of Benedict and Talbot[4] in the case of normal infants, and

[4] Benedict, F. G., *Boston M. and S. J.*, 1919, clxxxi, 107; Benedict, F. G., and Talbot, F. B., "Metabolism and Growth from Birth to Puberty," Carnegie Inst. Wash., Pub. 302, 1921.

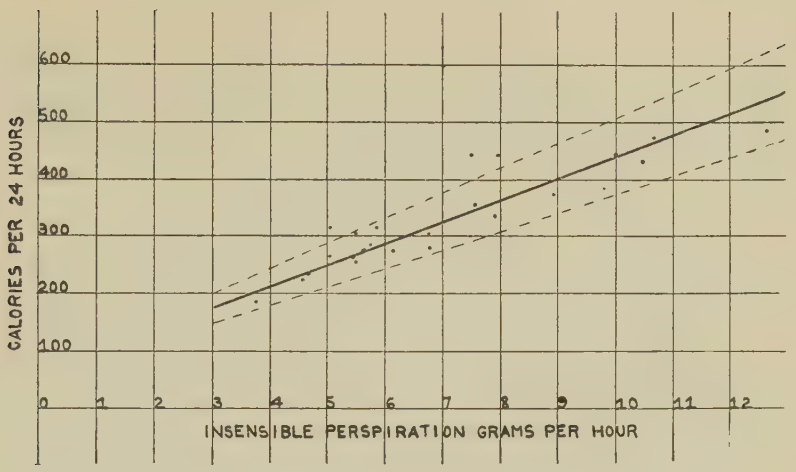

CHART 2.

Shows the same relationship as Chart 1. Insensible perspiration measured by the method of weighings, and the heat production determined in the respiration chamber or estimated from standard tables. The straight line curve shown in Chart 1 also represents the general trend of these points and is suggested as a method for predicting the metabolism of infants from the measured insensible perspiration.

from the average figures previously compiled by us[5] in the case of marasmic infants. Chart 2, arranged similarly to Chart 1, graphically shows the results. Each point on the chart represents the insensible perspiration as obtained by the method of weighings together with either the actually determined or estimated basal metabolism.

A straight line curve drawn through the scatter of points duplicates precisely the arbitrary curve constructed in Chart 1 from the results obtained in the respiration chamber experiments. Only 3 of the 23 points representing individual observations deviate from the curve by more than 15%. It seems probable, therefore, that the values for insensible perspiration obtained by the simple method of weighings may serve as an index for predicting the metabolism of infants. In view, however, of the relatively few observations thus far made on infants with this method and because of the significant deviations which have occasionally been found, the particular straight line curve suggested for predicting the metabolism of infants from the measured insensible perspiration must await confirmation before adopting it finally for purposes of prediction. With further refinement in the method and with added investigation it is possible that the slope of the curve may require slight change or

[5] Levine, S. Z., Wilson, J. R., and Gottschall, G., *Am. J. Dis. Child.* In press.

that deviations may be even further reduced. Studies are now in progress to test the reliability of the method and to determine the effect of various factors, such as food, visible perspiration, activity, body and environmental temperature and humidity on the insensible perspiration of infants.

3949

Rate of Development of Pneumococcus Immunity.

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Inasmuch as pneumonia is a self-limited disease ending by crisis or lysis generally between the 7th and 10th days, 3 to 5 days are frequently afforded in which an attempt may be made to produce active immunity before the natural termination of the disease takes place. At the suggestion of Dr. A. R. Dochez a study was therefore undertaken of the onset and rate of development of pneumococcus immunity.

The development of pneumococcus immunity after the introduction of pneumococcus vaccine has been studied from many points of view. The onset of immunity has been noted from the 5th to the 14th day by different workers,[1] in a few observations as early as the 3rd day. Many preparations of the antigens have been employed. Some workers[2] have believed the intact cell necessary for the production of well-marked type-specific immunity, whereas others have found that extracts or solutions of the cells were equally effective.[3] As we were interested in the preparation of a vaccine that would be especially effective in initiating early immunity, we compared in this regard a vaccine made from the intact cell with that obtained from a watery extract of the cell.

Three antigens were employed (1) a vaccine made from the intact cell, (2) a Berkefeldt filtrate of shaken bacteria, (3) a Berkefeldt filtrate of the broth culture. The method of preparation was as follows:

A pneumococcus type 11 organism of high virulence was grown for 6 hours in 2.5% human serum beef-infusion broth. This was used to inoculate 250 cc. flasks of similarly prepared broth in the

* With the technical assistance of Mr. Max Soroka.

proportion of 0.1 cc. inoculum to 5 cc. broth. At the end of 11 hours' incubation, part of the broth culture was centrifuged and the supernatant fluid passed through a Berkefeldt filter, the so-called broth filtrate. A second portion of the broth culture was centrifuged, and the supernatant fluid discarded. The sedimented bacteria were kept intact by rinsing the test-tube carefully with distilled water, removing traces of broth. The bacteria were then taken up in distilled water, to make a concentration of 1 billion organisms to 1.0 cc. The suspension was divided into 2 parts. One was heated to 60° for 1 hour, allowed to cool and tricresol added (final solution 0.3%)—the serum-vaccine. The second part was shaken by hand for 5 minutes, centrifuged, and the supernatant fluid passed through a Berkefeldt filter. It consisted of a clear watery solution, free from formed elements, sterile on culture, and was called the filtrate of the shaken bacteria or the bacterial filtrate.

Active immunity experiments were conducted by injecting a series of mice intraperitoneally with the 3 antigens and testing their resistance to a virulent culture from 1 to 7 days after inoculation. The dose of the vaccine was 200 million organisms, of the filtrates that amount of solution which represented previous contact with 400 million organisms. The test culture employed was fatal to a mouse in dilution of 10^{-6} cc.

The onset of definite immunity occurred on the 3rd day after inoculation of the vaccine, on the 4th day after the broth filtrate and the bacterial filtrate. In all instances the immunity increased markedly to the 5th day, and remained approximately stationary to the 7th day. On the 5th day the mice withstood an injection of 0.001 cc. of culture. In some animals this degree of resistance was evident on the 3rd day after inoculation. The degree of active immunity which the mice developed on the 5th day protected against 10,000 minimal lethal doses of virulent pneumococci, in some instances, particularly when Type 1 was employed as an antigen, 100,000 minimal lethal doses. In order to prove that the immunity produced was type-specific, the mice who survived were given an equivalent dose of Type I test-culture of the same virulence. None survived. In addition, a series of mice were immunized by the above 3 antigens derived from Type II and given a test-culture of Type I on the 3rd, 4th, and 5th days after inoculation. None survived.

Passive immunity experiments were conducted by injecting rabbits with the above antigens. Their sera protected mice in the same degree as that obtained in the active immunity experiments with the exception that individual rabbits varied in their capacity to produce protective substance. Tests were made to determine whether sera

which protected against the homologous organism had any protective power against the heterologous organism (Type I) with completely negative results.

Two conclusions may be drawn from these experiments: (1) Active and passive immunity of considerable degree may be produced 3 days after injection of a suitable vaccine derived from the intact organism, Type I or Type II. The immunity increases markedly to the 5th day and remains approximately stationary to the 7th. In the case of the filtrates either from the broth culture or the shaken bacteria, the immunity begins on the 4th day, increases on the 5th day and remains stationary to the 7th day. (2) The immunity produced during this period was not due to the elaboration of the common protein antibody but to the development of type-specific protective substances. This was equally true after the injection of an extract of the pneumococcus obtained by simple Berkefeldt filtration as well as after injection of the intact cell.

The study was undertaken primarily with the object of determining whether an active immunity to the pneumococcus could be established in a sufficiently short space of time as to make the injection of vaccine a therapeutic possibility in lobar pneumonia. As far as the time interval is concerned our results support this hypothesis. Whether the patient with lobar pneumonia would react by an earlier initiation of his immunity mechanism is not within the scope of this paper.

3950

A New Phenomenon of Local Skin Reactivity to B. Typhosus Culture Filtrate.

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From the Laboratories of the Mount Sinai Hospital, New York City.

A phenomenon of local reactivity to *B. typhosus* culture filtrate is described. The work was done on rabbits. The reactivity was induced by skin injections of the filtrate 24 hours prior to the intravenous injection of the same filtrate. The local reactions appeared at the site of previous skin injections; they were fully developed in 4 to 5 hours after the intravenous injection; they were extremely severe and microscopically showed very pronounced necrosis of tissue with rupture of blood vessels and extensive local hemorrhage. The reactions in different rabbits varied in size from 1x1 cm. to 4x4 cm.

The phenomenon was reproduced in several hundred rabbits. About 78% of animals tested showed locally severe hemorrhagic reactions. The remaining animals were spontaneously resistant to the phenomenon.

When several areas of the skin of the abdomen were prepared by skin injections the intravenous injection produced reactions in each prepared area. The reactions were uniformly severe. The only variation in the response of different areas of the skin of the same animal was in the size of the reactions. The average difference was from 1 to 2 cm.

The skin injections themselves produced an erythema. The intensity of the erythema varied in different animals and in various areas of the skin of the abdomen of the same animal. The intensity and size of local hemorrhagic reactions to the intravenous injections was not related to the intensity of the erythema produced by the preparatory skin injections. Very severe hemorrhagic reactions were also produced by intravenous injection in areas which reacted negatively to the preparatory skin injections.

For the reproduction of the phenomenon there was necessary an interval of a few hours between the skin and intravenous injections. An incubation period of 2 hours was insufficient. An interval of 24 hours was invariably sufficient. The ability to react disappeared in 48 hours after the preliminary skin injection.

Repeated injections of the filtrate into the same area of the skin with an interval of 24 hours between the injections did not result in reactions similar to the above described hemorrhagic necrosis. The second skin injection was followed by reddening, some swelling and migration of polymorphonuclear neutrophile leucocytes which showed no signs of necrobiosis. There was no rupture of blood vessels. Skin injections followed after a suitable interval by intravenous injections were necessary for the reproduction of severe local response.

In several experiments the preparatory skin injection of *B. typhosus* culture filtrate was substituted by local injections of turpentine in various doses and filtrates of culture of various strains of streptococci. No local response followed the intravenous injection of *B. typhosus* culture filtrate into animals prepared in this manner.

Studies on the relation of specific anti-sera to the phenomenon described are under progress.

In a further communication local skin reactivity to filtrates of cultures of microorganisms other than *B. typhosus* will be described.

3951

The pH of the Blood of Chicken Embryos.

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From the Hospital of the Rockefeller Institute for Medical Research.

In continuing our work on physiological ontogeny we have thought it of value to study the variation with time of the hydrogen ion concentration of the blood of chicken embryos. Estimates were made on embryos from 7 days of age to term. In making the measurements we used glass electrodes. We have taken precautions on the score of injury, the changes which take place during the process of dying, glycolysis and of temperature, the measurements being carried out in a warm room between 38 and 39° C. The usual precautions in the handling of blood have also been taken.

We find that at 7 to 8 days the blood is, relatively speaking, acid, and from 9 to 15 days maintains a rather level course somewhat more alkaline. At about the 16th day, the blood becomes still more alkaline and approaches the value found in adult chickens. We have studied both arterial and venous blood and mean to report upon these experiments in detail at a later time.

We have also taken blood from the foetuses of cats. From these we obtained figures indicating the presence of the same order of acidity. The data seem to indicate that the values are characteristic of embryonic growth in general and are not peculiar to the method of development, that is to say, of eggs versus foetuses.

3952

Parasites in Artificial (Inoculation) Malaria.

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From the Bacteriological and Clinical Departments of the Psychiatric Institute, Ward's Island, N. Y.

The treatment of general paralysis by inoculation with malaria has been employed for the past five years at the Psychiatric Institute.[1] More than 300 patients with general paralysis have been inoculated intravenously with a single strain of malaria which has been therapeutically effective. The present results are concerned with:

[1] Bunker, H. A., and Kirby, G. H., *J. Am. Med. Assn.*, 1925, lxxxiv, 563.

1. A detailed microscopic study of the life-cycle of this parasite made by blood smears taken hourly from 4 patients inoculated with malaria about 3 years ago.

2. The significant finding was the total absence of gametocytes (the sexual forms of the malarial parasite). This agrees with contentions of Gerstmann.[2] The asexual cycle of this strain of malaria is identical with the asexual cycle of "natural" malaria. From a biological point of view it is interesting to note that our malarial strain has apparently become "sterile", which means that it has lost its capacity for sexual propagation. The absence of gametocytes would make it impossible to infect mosquitos, thus limiting the life-cycle to man alone. Incidentally, we have failed in our efforts to cultivate this strain of malaria in artificial media.

3. A strain of malaria free from gametocytes is of considerable practical value in the treatment of general paralysis in that:

(a) It eliminates the possibility of the transmission of malaria to other members of the community.

(b) It precludes the occurrence of malarial relapse following adequate quinine administration.

3953

Spectrophotometric Analysis of Dye Penetrating Nitella from Methylene Blue.

MARIAN IRWIN.

From the Laboratories of the Rockefeller Institute.

When living cells of *Nitella* are placed in methylene blue solution the rate of penetration of dye into the vacuole is more rapid at pH 9.2 than at pH 5.5. The penetration at pH 5.5 is too slow for satisfactory spectrophotometric analysis but at pH 9.2 this is possible. The dye in the vacuole gives an absorption curve characteristic of a dye consisting chiefly of azure B and a trace of methylene blue with an absorption maximum at 655mμ, while the external solution gives a curve characteristic of methylene blue with an absorption maximum at 664mμ. The presence of methylene blue in the sap is not due to contamination because the sap is extracted by cutting the end of the cell which is wrapped in dampened absorbent cotton and kept

[2] Gerstmann, S., *Die Malariabehandlung der Progressiven Paralyse.* J. Springer. 1925.

outside the solution. The same result is obtained whether the analysis is made immediately or several hours after extraction of the sap from the vacuole.

This result shows that the dye in the sap of *Nitella* has more methylene blue than that of *Valonia* (the primary absorption maximum of the latter being at 650mμ). This difference may be due either to the difference in the conditions of cells brought about by experiments or due to the difference normally existing. As soon as cells are injured more methylene blue penetrates.

The sap of *Nitella* is incapable of changing methylene blue to azure B even in 20 hours, when a sample of methylene blue is dissolved in the sap.

The rate of penetration of dye from pure azure B solution at pH 9.2 is much more rapid than from methylene blue solution at pH 9.2. The dye which has penetrated is found to be azure B with an absorption maximum of 650 mμ, which is identical with that of the external solution. At pH 5.5 the penetration is too slow for satisfactory analysis. These results agree with those on *Valonia*.

These results support the theory that the dye which penetrates the vacuole of living cells rapidly is the one which is quickly absorbed by the non-aqueous layer of the protoplasm and extracted from this non-aqueous layer by the sap. Azure B penetrates at a greater rate than methylene blue at pH 9.2, because in the form of free base it is rapidly absorbed by the non-aqueous layer. It is rapidly extracted by the sap on account of the fact that as soon as it comes in contact with the sap (which is at pH 5.5) it is transformed to salt, which is not readily absorbed by the non-aqueous layer. Since the dye salt cannot diffuse out it accumulates in the sap. Methylene blue which exists only in the form of salt, on the other hand, is only slowly absorbed from the solution at pH 9.2 by the non-aqueous layer and given up to the sap. The rate of penetration, therefore, depends on the partition coefficient of each form of dye at each respective boundary between the non-aqueous and aqueous phases.

These results do not show that methylene blue does not penetrate the protoplasm. They merely point out the danger of drawing any theoretical conclusion from such experiments unless we are certain of the nature of the dye penetrating.

3954

Precipitable Substances Prepared from Bacilli of the Salmonella Group.

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From the Laboratories of The Rockefeller Institute for Medical Research.

Extending previous work,[1] soluble specific substances were prepared from a number of types of the Salmonella group which yield large amounts of reducing sugar on hydrolysis, and are resistant to tryptic and peptic digestion and to the action of alkaline hypochlorite solution. The group reactions of these substances correspond in a general way to those of the so-called stable agglutinogens[2] regardless of the method in which the extracts were prepared. (Digestion of the autoclaved bacilli with trypsin, or extraction of the alcohol treated bacilli by heating with saline solution, or dissolving the microbes with alkaline hypochlorite solution.) This parallelism was also seen when the specificity was tested by means of immune sera absorbed with heterologous bacilli of the Salmonella group.

TABLE I. *Precipitation tests.*

Precipitable substances (crude preparations)		Immune sera prepared with bacillary suspensions of the serological types						
Obtained from	Dilution 1	Typhosus	Enteritidis	Paratyphosus B	Stanley	Reading	Newport	Suipestifet
Typhosus	2000	+++	++	0	0	±	±	tr.
	20000	++	+±	0	0	±	0	±
Enteritidis	2000	+++	++±	0	0	0	0	0
	20000	++	++	0	0	0	0	0
Paratyphosus B	2000	++	++	+++	+++	++±	f. tr.	0
	20000	+	++	++	+±	+++	0	0
Stanley	2000	0	++±	++	++	++++	0	0
	20000	0	+±	++±	++	+++	0	0
Reading	2000	tr.	++	+++	+++	++±	0	f. tr.
	20000	0	±	+±	±	+	0	0
Newport	2000	0	0	++	+±	0	++++	0
	20000	0	0	+±	+	0	++	0
Suipestifer	2000	0	0	0	0	0	+++	+++
	20000	0	0	0	0	0	+++	++±

0.2 cc. of the antigen dilutions plus 1 drop of immune serum. The test tubes were kept for 2 hours in the room and over night in the ice box.

[1] Landsteiner, K., and Furth, J., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 379, 602, 771; *J. Exp. Med.*, 1928, xlvii, 171.

[2] White, P. B., Med. Research Council, Special Report Series, 1926, 103.

One striking exception to this was observed with the soluble substance of *B. paratyphosus B* and *Stanley*. The sera for these types when absorbed with bacilli of the strains *Reading* or *Abortus Equi* no longer precipitated the homologous soluble specific substance, although they still agglutinated the homologous bacilli, a reaction in which factor I (White) is involved. It was found that this loss was due to a high sensitivity to alkali of the precipitable substance as originally extracted by saline solution. On the contrary, the other reactions mentioned persisted even after boiling the preparation for several hours with normal sodium hydroxide, whereas hydrolysis with normal hydrochloric acid for a few minutes resulted in liberation of reducing sugar and the destruction of the serological properties. The reactions for factor I were not altered by digesting the substance with pepsin or trypsin at a pH which in itself was not injurious.

It was attempted to establish whether or not by precipitation with immune sera various fractions could be separated from the precipitable substance of one bacillus as one would expect if a special substance would correspond to each single serological factor. In these tests the sera were properly absorbed with heterologous bacilli in order to prepare antibody solutions active for certain factors only. The washed precipitates were dissolved by boiling for a few seconds with dilute alkali. On the whole the results did not indicate a separation of antigen fractions by this method except perhaps in one case. Such a separation, however, could easily be accomplished under the same conditions when a precipitate was made with a mixture of substances derived from different bacilli.

The specific substances of *B. paratyphosus B* and *B. typhosus* reported on previously could be further purified. The preparation recently obtained from *B. paratyphosus B* yielded on hydrolysis with n/2 HCl for 5 h. 77.3% reducing sugar (calculated as glucose) and contained 0.66%. The corresponding substance from *B. typhosus* containing 1.02% N gave 73.2% sugar on hydrolysis. This preparation was precipitated by a common typhoid immune serum up to 1 :1,000,000. On hydrolysis of the above substances some insoluble material separated out.

By immunization with small amounts of the preparation described at P2 typhosus[1] immune sera were obtained which agglutinated typhoid bacilli. After removal of the small flaking agglutinins by absorption with typhoid bacilli heated to 100° C. for 2 hours this agglutination had the character of that produced by large flaking agglutinins. Sera with similar properties were produced by injection of typhoid bacilli boiled with alcohol. In both instances

the large flaking agglutinins differ somewhat from those of common typhoid immune sera.

The substance P_2 was found to be highly toxic for rabbits and rats.

3955

Relation of Cholesterol and Lecithin to Remission in Pernicious Anemia.

GULLI LINDH MULLER, WILLIAM B. CASTLE, EMILIE GOODE AND MIRIAM ROSE.

From the Thorndike Memorial Laboratory of the Boston City Hospital.

It has been recognized that the cholesterol and lecithin content of the blood are low in severe relapses of pernicious anemia and that these constituents tend to increase with the improvement of the patient's health. Whether the increase of cholesterol and of lecithin bears any definite relationship to the remission of the anemia has not been determined.

Determinations of cholesterol and lecithin in the plasma of 25 cases of typical pernicious anemia have been made, usually every other day over a period of many weeks. Cholesterol was determined by Bloor's method[1] and the lecithin according to the method described by Whitehorn.[2] On admission 4 cases had less than 1.0 million red blood cells, 7 had between 1.0 million and 2.0 million, and the other 14 had over 2.0 million per cu. mm. Of the 4 cases with less than 1.0 million red blood cells per cu. mm., all showed low values for both cholesterol and lecithin; in some instances less than 50% of normal. Two of these 4 cases had a rapid remission, one died soon after entering the hospital, and the fourth is still under observation.

The first case entered the hospital with a red blood cell count of 950,000 per cu. mm. The plasma cholesterol was 93 mg., and the lecithin phosphorus 4.3 mg. per 100 cc. For 17 days the patient was fed a test preparation. During this time the red blood cells fell to 644,000 per cu. mm., the cholesterol fluctuated between 56 and 93 mg. per 100 cc., while the lecithin phosphorus varied between 4.3 to 5.7 mg. per 100 cc. The patient was then given another test preparation of the same cholesterol content. A remission promptly

[1] Bloor, W. R., Pelkan, K. F., Allen, D. M., *J. Biol. Chem.*, 1922, lii, 191.

[2] Whitehorn, J. C., *J. Biol. Chem.*, 1924, lxii, 133.

set in, and a typical response of the reticulocytes occurred, accompanied by a considerable rise in the cholesterol and lecithin values. As the red blood cells and the hemoglobin rose the cholesterol and lecithin increased still further and approximately normal values were reached when the red blood cells had increased about 1.0 million per cu. mm.

A second case during a 9 day control period was fed with an inert preparation, and the red blood cells remained about 700,000 per cu. mm.; the plasma cholesterol varied from 48 to 91 mg. per 100 cc., and the lecithin phosphorus from 3.9 to 5.0 mg. A transfusion of 500 cc. of blood was given on the ninth day and the patient placed upon a daily dose of a liver extract preparation. Directly after the transfusion, the red blood cells rose from 600,000 to 1,500,000 per cu. mm. without any appreciable rise of the cholesterol and the lecithin. On the seventh day after commencing the liver extract, the reticulocytes reached the peak of their rise which indicated the onset of remission. This was accompanied by an increase of both cholesterol and lecithin which reached 101 and 6.2 mg. per 100 cc. respectively, when the red blood cells numbered only 1.3 million per cu. mm.

The observations in these 2 cases of pernicious anemia, which are comparable to those upon others, indicate that cholesterol and lecithin increase in the plasma coincident with the onset of remission. The increase is evidently not dependent upon the actual number of red blood cells in the circulation. Whatever fundamental changes occur with the onset of the remission in pernicious anemia, the increase of cholesterol and lecithin seems to be one phase of this change.

3956

Permeability of the Fundulus Egg to Ions: Chorion versus Skin.

MARGARET SUMWALT. (Introduced by M. H. Jacobs.)

From the Department of Physiology, University of Pennsylvania Medical School, and the Marine Biological Laboratory, Woods Hole, Mass.

In previous studies on the permeability of the Fundulus egg, little attention has been paid to the fact that the embryo is enclosed by 2 membranes. One of these is the skin of the embryo itself; or, at very early stages before the blastodisc has completely enclosed the yolk, the delicate vitelline membrane which covers the yolk. The

other is the chorion, the noncellular, flexible, inelastic outer shell, which has no organic connection with the embryo, but retains a cushioning fluid layer about it. Some of the criteria of permeability used in the previous work on Fundulus eggs, such as cessation of the heartbeat, and coagulation of the embryo, by acid penetration, may have required that both membranes be traversed by the substance studied. Others, of which Loeb's specific gravity test is a type, probably involved only the chorion. But in none of the earlier papers have data been presented for a satisfactory comparison of the permeabilities of the 2 membranes.

It was thought that evidence bearing on this question might be obtained by a method which Michaelis has recently applied to the study of the permeability of certain non-living membranes, such as dried collodion. Michaelis has described the permeability to ions of these membranes in terms of concentration potentials measured across the membranes between solutions of N/10 and N/100 KCl. This general method with certain modifications has been applied to the Fundulus egg material by measuring E.M.F. across the membranes of a single egg from inside to outside. The egg was impaled on a capillary pipette which, in connection with a calomel half cell made up in sea water, constituted the inside electrode; and was immersed in a solution into which dipped the outside electrode, of similar construction. The electrodes were made up with sea water rather than KCl in order that the solution introduced into the egg with the capillary electrode might be a physiologically balanced mixture. Experiments with sea water half cells in a non-living system in which saturated KCl half cells could also be used, indicate that the diffusion potentials introduced into the E.M.F. measurements by the former largely balance out, and that the small residual error is insignificant when it is the difference between the two kinds of concentration potentials which is being considered. Since the egg contents had to be maintained constant, the concentration effect was secured by exposing the outside of the egg successively to 2 concentrations of the same solution. In the present paper, the algebraic difference between the potentials observed when the egg was in undiluted sea water and in sea water diluted 100 times, is termed the concentration potential. The movements of the inside electrode were governed by a Chambers micromanipulator and observed through the low power of a microscope. For a measurement across the chorion alone, the electrode tip penetrated the chorion and lay in the subchorionic fluid; for a measurement across chorion and embryonic skin together, it was thrust into the yolk sac of the embryo.

The results of these measurements showed that the compound membrane of skin plus chorion was capable of producing concentration potentials much greater than those produced by the chorion alone. The average of the chorion values was 19.4 mv. with a maximum of 40.5 mv., as compared with the figures across chorion plus skin, which averaged 55.2 mv. and reached an extreme of 114.6 mv. For both sets of values, with change to the dilute solution, the inside of the egg became more negative to the outside. If it is permissible to interpret these results in the same manner as those of Michaelis on simpler membranes, the negative sign of the concentration potential indicates a relatively greater impermeability of the membranes to anions than to cations. And its greater magnitude in the case of the 2 membranes together, than in that of the chorion alone, furnishes evidence that the differential permeability is less pronounced in the chorion than in the system as a whole.

That the relative impermeability to anions of the system as a whole is largely due to the skin is indicated by measurements of electrical resistance. When allowance was made for the resistance of the remainder of the system, the net resistance of the chorion to direct current was found in 23 experiments to range from 7,000 to 92,000 ohms. On the other hand, the resistance of the chorion plus the skin was 300,000 ohms, or more, in all cases. Since resistances in series are summed, it would appear that the resistance of the skin alone was, in these cases, at least 208,000 ohms.

3957

Preservation of Luminous Bacteria in Absence of Oxygen.

CHARLES S. SHOUP. (Introduced by E. N. Harvey.)

From the Physiological Laboratory, Princeton University.

In the course of experiments on the respiration of the luminous bacterium, *Bacillus fischeri*,[1] it became necessary to determine whether or not this organism was a facultative anaerobe, a characteristic assigned by Beijerinck[2] to one species of luminous bacteria.

The luminescence of luminous bacteria is absolutely dependent on the presence of a partial pressure of oxygen in the medium or

[1] Migula, W., *System der Bakterien.* Jena, 1900.

[2] Beijerinck, M. W., *Arch. Neerlandaises,* 1889, T. 23, 416.

the atmosphere in which they grow.[3, 4] If all oxygen is removed from the medium, and from the atmosphere above the medium after inoculation, the organisms may be allowed to remain in quite anaerobic conditions. Preparations were made for the maintenance of luminous bacteria in absence of oxygen as follows:

A number of experimental tubes were made by narrowing the middle of the ordinary bacteriological culture tubes to facilitate sealing off after inoculation. Nutrient media was placed in the sterile tubes and the whole autoclaved. By means of short and long glass tubes passed through the tightly-fitting rubber stopper replacing the cotton plugs in the tubes, pure hydrogen was passed over the medium and out again. (It is necessary that this hydrogen be passed over hot platinized asbestos contained in a heated quartz tube and be carried to the preparation through lead tubing to prevent diffusion of oxygen into the system.) The medium was boiled to eliminate oxygen while the pure hydrogen passed through the system, cooled as a slant with the pure hydrogen passing over the medium, quickly inoculated with a vigorous growth of the luminous bacteria and replaced in the system so that the pure hydrogen might again replace all air present. With the pure hydrogen still passing rapidly through the system, the tube was then sealed off at the narrow neck, allowing the inoculation to remain in an atmosphere of pure hydrogen, and on an oxygen-free medium. A number of tubes prepared in this manner were set aside with two opened to the air and plugged with sterile cotton to serve as controls.

Within 12 hours an abundant growth with brilliant glow appeared in the control tubes, where air was allowed to replace the hydrogen atmosphere. After from 48 to 72 hours no growth had occurred in the tubes where inoculations were maintained in an atmosphere of pure hydrogen, but on opening these at various intervals a good growth with brilliant luminescence appeared within 12 hours. Inoculations made in the above manner on oxygen free-media and sealed in atmospheres of pure hydrogen have been kept for periods exceeding 2 months (64 days) before opening, and have always regained luminescence and have begun to grow abundantly within 12 hours after the tubes were opened to an atmosphere of air. The inoculation itself has been in every case preserved and kept moist, yet no actual growth occurs so long as an atmosphere of pure hydrogen is maintained above the medium. The property of luminescence is quickly regained on readmission to the normal atmosphere, no drying having impaired the vigor of the inoculation, and

[3] Harvey, E. N., "The Nature of Animal Light," Philadelphia, 1920, p. 67.

[4] Harvey, E. N., *Physiol. Rev.*, 1924, iv, 4, 639.

a complete new growth may shortly be obtained for reinoculation.

It has been shown that the amount of oxygen necessary for just visible luminescence in a suspension of luminous bacteria in sea-water is only of .0053 mm. of mercury pressure.[5] The amount of oxygen in tubes sealed in pure hydrogen with nutrient medium out of which dissolved gases have been driven by boiling, must be very minute indeed.

It has been demonstrated that oxygen is necessary for the growth of this species of luminous bacterium, *(B. fischeri)* as well as for the maintenance of luminescence. The very fact that the glow reappeared in the experimental tubes in each case only after several hours of exposure to an atmosphere containing a partial pressure of oxygen, indicated that it was necessary for growth to occur and permit luminescence over a larger area of medium before it became visible to the observer.

The above method is also suggested as a means of storage and transport of the living luminous bacteria where one is especially anxious that luminescence reappear quickly. The inoculation itself while in pure hydrogen will lie dormant on the nutrient medium until again exposed to an atmosphere containing a partial pressure of oxygen sufficient for growth and luminescence, whereupon both occur, and a vigorous culture of the organisms will cover the surface of the medium.

3958

The Presence of an Unknown Factor in Serum Which Influences Calcification.

L. EMMETT HOLT, JR., AND PAUL G. SHIPLEY.

From the Department of Pediatrics, Johns Hopkins University School of Medicine.

It has been shown by Shipley[1] and the observation has been confirmed by Shipley, Howland and Kramer[2] that calcification of slices of cartilage and bone from a rickety animal takes place *in vitro* within 48 hours when the slices are immersed in normal blood serum at 37° C.

In the course of a series of experiments upon calcification from

[5] Harvey and Morrison, *J. Gen. Physiol.*, 1923, vi, 13.

[1] Shipley, P. G., *Johns Hopkins Hosp. Bull.*, 1924, xxxv, 304.

[2] Shipley, Kramer and Howland, *Trans. Am. Ped. Soc.*, 1925, xxxvii, 36; *Am. J. Dis. Child.*, 1925, xxx, 37; *Biochem. J.*, 1926, xx, 379.

solutions of inorganic salts, we have repeatedly observed that calcification takes place only when the concentrations of blood electrolytes are considerably reduced below the concentrations in the blood serum. When a salt mixture is used approximating as closely as possible the inorganic composition of blood serum,[3] no calcification whatever occurs, even in preparations which have been incubated as long as 10 days. A sample experiment is given in the table. It would therefore seem necessary to postulate the existence in the blood serum of some factor which enables calcification to take place in spite of the high concentration of electrolytes.

TABLE I.

Medium	Bone	Number of Preparations	Time	Calcification
Normal Serum	Rat	6	48 hours	Positive
Salt Solution:				
Na 120.0 mM.				
K 0.3 mM.				
Ca 2.2 mM.	Rat	15	48 hours	Negative
Mg 0.8 mM.				
Cl 90.0 mM.	Rat	3	2 weeks	Negative
CO_3 32.0 mM.				
P 1.33 mM.				
SO_4 0.8 mM.				

It seems altogether improbable that the serum proteins could be concerned in producing this effect, since proteins in general, owing to their ionic nature, would be expected to inhibit rather than to promote calcification. Moreover, the experiments of Shipley, Howland and Kramer have shown that egg albumen and gelatin actually do inhibit the process.

Experiments to determine the nature of this unknown factor are now in progress.

[3] Shipley and Holt, *Johns Hopkins Hosp. Bull.*, 1927, xl, 1.

3959

Factors Concerned in Demonstrating Utilization of Carbohydrates by Organisms of the Genus Mycobacterium.

MALCOLM H. MERRILL. (Introduced by M. S. Fleisher.)

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In plain broth cultures of organisms of the genus *Mycobacterium* I have shown that the method of incubation definitely affects the reaction changes produced. Cultures sealed from the atmosphere were shown to produce only slight growth with an accompanying slight drop in the pH of the media. This slight growth was shown to be due to lack of oxygen and the slight reaction change to the acid side has been shown to be due to production and retention of carbon dioxide. Cultures not sealed from the atmosphere were shown to become progressively alkaline.

Reaction change determinations, using a modification of the method of Long and Major,[1] upon mannite, galactose, and dextrose broth cultures of 9 organisms of this genus showed only 3 outstanding variations from the plain broth control. These 3 were as follows: *Myco. leprae* and *Myco. phlei* in mannite broth and *Myco. chelonei* in dextrose broth.

These results, as well as others, showed definitely that the primary change in reaction is not to the acid side when these organisms are grown in carbohydrate broth. The suggested explanation of the peculiarly characteristic action of organisms of this genus when grown in carbohydrate broth was that these organisms utilize the carbohydrates completely, no cleavage products remaining in the media. This characteristic is undoubtedly closely associated with the strict aerobic nature of these organisms. It was concluded that the method of determining carbohydrate utilization by noting the appearance of acid in the media, which is the method generally used for such determinations, is not applicable to the determination of carbohydrate utilization by organisms of the genus *Mycobacterium.*

Furthermore, the reaction curves are influenced to such a slight degree by the addition of carbohydrates to the plain broth, regardless of whether or not the carbohydrates are utilized, that reaction curve determinations constitute an indefinite and impracticable method of determining carbohydrate utilization by organisms of this genus.

[1] Long, C. R., and Major, A. L., *Am. Rev. Tuber.*, 1922, v, 715.

It was noted that the general tendency was for the cultures to increase in pH with less rapidity in the presence of carbohydrate. This was attributed to the protein sparing action of carbohydrates.

3960

Clinical Pathology and Bacteriological Study of Non-erupted Mandibular Molars.*

FRANK HARUYUKE ITO. (Introduced by John Albert Marshall.)

From the Department of Biochemistry, University of California Medical School.

This report suggests a classification of the various lesions of soft tissues produced by the difficult eruption of mandibular third molars. In addition it presents a bacteriological study of dental pulps dissected from embedded teeth which were extracted.

Unerupted teeth are classified as either impacted or embedded depending upon their relation to adjacent teeth and overlying bone. Both types may produce neuralgia and other lesions of nerve, muscle or organs of special sense. The local inflammatory reactions are classified under 4 general heads: (1) Simple pericoronitis; (2) Supurative pericoronitis (acute and chronic); (3) Diffuse pericoronitis; (4) Hypertrophic or hyperplastic pericoronitis. These several conditions require certain precautions in preliminary treatment but call ultimately for the removal of the tooth.

The bacteriological study of the vital pulps of unerupted third molars is a virgin field. The organisms of dental caries and pyorrhea have been extensively studied but no report has been published so far as we have been able to discover, of a bacteriological research upon dental pulps of embedded teeth. Only teeth with unbroken or non-mutilated crowns and roots were utilized. The technic of sterilization of tissues and instruments before and after extraction was carefully checked against accidental contamination from bacteria.

From an examination of embedded and impacted teeth where pericoronitis had developed it was found that over three-fourths gave cultures of non-hemolytic green producing streptococcus. This relative frequency is probably due, first, to the fact that unerupted third molars have larger apical foramina than erupted teeth. Thus, there is greater opportunity for organisms to gain access to the pulp.

* Submitted in partial satisfaction for the requirement of Master of Science degree, University of California.

Second, the pouch of the follicle which is attached to the neck of the unerupted tooth is very susceptible to infection. Nevertheless, it is pointed out that in some cases pericoronitis may exist for years without causing acute symptoms. At a later time conditions may develop which favor the development of the organisms. Furthermore, a higher degree of virulence may arise and thus a latent infection may flare up into an acute one.

3961

Treatment of Rickets With Irradiated Ergosterol (Vigantol).

SAMUEL KARELITZ. (Introduced by B. Schick.)

From the Pediatric Department, Mount Sinai Hospital, New York City.

The absence of any clinical report of the effect of irradiated ergosterol on human rickets in the American literature seemed to justify recording the results obtained in 9 cases of rickets treated with Vigantol. Both the German product and the first American product were used. The latter was prepared by the Winthrop Chemical Company. Hess[1] has already reported successful results in experimental rat rickets with ergosterol and in human rickets through the use of irradiated yeast.

The researches of Windaus, Hess, Pohl, Rosenheim,[2] and their co-workers established the fact that ergosterol is the provitamine which becomes Vitamine D after irradiation for ½ to 12 hours. If irradiated longer, its power of being activated is gradually lost, being completely lost after 80 hours of exposure to ultraviolet rays. It is obtained from yeast, ergot, and mushrooms, and occurs as a contaminator of cholesterol from 1/20 to 1/50%. It is oxidized if exposed to air for a long time, but if kept in oil (as Vigantol) it retains its potency for at least 6 months.

Rosenheim, Gyorgy,[3] Hottinger,[4] and others have shown that irradiated ergosterol is 20,000 times as antiricketic as cod liver oil and that in dosage of 1/10000 mg. daily it prevents rickets in a rat on a ricketic diet, and that 1-5 mg. daily heals human rickets, osteomalacia, tetany, and rickets tarda and is also effective prophylactically in

[1] Hess, A. F., *J. Am. Med. Assn.*, July 30, 1927.

[2] Rosenheim, O., and Webster, T. A., *Biochem. J.*, 1927, xxi, 127; *Lancet*, Feb., 1927.

[3] Gyorgy, P., *Klin. Woch.*, 1927, xiii, 580.

[4] Hottinger, A., *Z. f. Kinderheil*, 1927, xliii, 8; 1927, xliv, 3.

premature infants. The reports in the German literature are numerous and complete. Recent work[5, 6] here and abroad shows that the purified cholesterol still after irradiation has antiricketic properties if given in a dosage 30 times as great as originally used by Windaus and Hess. Vollmer[7] has shown that a single large injection of Vigantol, *i. e.*, 1 mg. ergosterol in a ricketic rat will cure the disease, thus showing that the vitamine can be stored and acts over a longer time.

As an example of the type of cases which we treated, one history is given in detail:

E. N., male, Italian, 7 months old, full term, breast fed 2 months, never received cod liver oil or ultraviolet ray treatments, was admitted because of pneumonia. Extensive occipital craniotabes, large parietal bosses, anterior fontanelle of 5x5 cm., a large rosary, Harrisons groove, flaring of the ribs, a pot belly and a palpable spleen, bow legs, enlarged wrists and ankles were present. Blood serum calcium was 9.5 mg. % and phosphorus 2.0 mg. %. X-ray picture showed marked enlargement of wrists with irregularly fringed and cupped epi-diaphyseal junction, a wide metaphysis and generalized poverty of lime. On 3.3 mg. of ergosterol daily in the form of Vagintol administered with milk he showed evidence of healing of the craniotabes in 12 days. The X-ray picture in 18 days showed an increase in lime of the long bones plus the deposit of white line of calcification at the epiphyseal end of the metaphysis. In 6 weeks the entire metaphysis was impregnated with lime and 2 new centers of ossification of wrist bones made their appearance. The blood serum calcium and phosphorus after 14 days changed to 9.8 and 4.2 mg. % respectively. The pneumonia improved without complications. The results in the other 8 cases were similar.

This exemplifies the typical result obtained in all but one of our 9 cases, both colored and white children. One very extreme case showed progressive healing but not a complete cure, even after 8 weeks. All but one child received 3.3 mg. ergosterol in oil (Vigantol) along with one of their milk feedings.

Craniotabes: Fastest time of healing—author, 7 days.
Fastest time of healing—other authors, 6 days.
Average time of healing—author, 14-21 days.
Average time of healing—other authors, 14-21 dys.

X-ray evidence of healing: Author—earliest, 17 days.
Other authors—earliest, 10 days.

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[5] Bills, Honeywell and MacNair, *J. Biol. Chem.*, 1928, lxxxvi, 261.

[6] Jendrossik, A., and Kemenyffi, A. G., *Biochem. Z.*, 1927, 189.

[7] Vollmer, H., *Z. f. Kinderheil*, 1928.

Author—average, 21 days.
Other authors—average, 21 days.

Complete healing: Author—6 weeks plus.
Other authors—5 weeks plus.

Return of calcium and phosphorus to normal: Author—14-21 days; other authors—14-28 days.

It is hoped that our dairies will be requested either to radiate the milk or preferably to add irradiated ergosterol so that we may use this newer knowledge to advantage in attempting prophylactically to prevent the extensive occurrence of rickets.

3962

Effect of Glucose on Urea Decomposition by B. Proteus.

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From the Department of Bacteriology, Northwestern University Medical School.

A typical strain of *B. proteus vulgaris* was grown in sugar free beef infusion broth, 1.0% glucose infusion broth, 0.5% urea infusion broth, and 1.0% glucose, 0.5% urea infusion broth, respectively. Incubation was practiced at 37° C. for 1, 3 and 5 days, at which times the following determinations were made:

a—Titratable acidity or alkalinity expressed as cc. of normal acid or alkali per 100 cc. of media.

b—Hydrogen-ion concentration.

c—Quantitative ammonia expressed as mg. of ammonia nitrogen per 100 cc.

d—Quantitative sugar expressed in percentage.

Results—For simplicity only the maximum readings without respect to days of incubation are here reported.

The reaction of the plain broth became 1.8 cc. alkaline, pH 7.3 to 8.3 and the glucose broth 3.0 cc. acid, pH 7.2 to 5.3. The amount of ammonia produced in the glucose broth was 8.9 mg. as against 36 mg. in the same broth without sugar. The alkalinity of the plain urea broth increased 6.7 cc., pH 7.3 to 9.2, and the ammonia 179.3 mg. The reaction of the glucose urea broth changed 1.5 cc. alkaline, pH 7.2 to 8.0, in contrast to the acidity of the glucose broth but not nearly to the degree of alkalinity manifested by the plain urea broth. Nevertheless 40.1 mg. more ammonia was produced in the glucose urea broth than in the plain urea broth,

which is still more significant in view of the fact that the sparing action of glucose for protein is here also undoubtedly operative. Clearly the presence of glucose accelerated the decomposition of the urea.

In the glucose broth the amount of glucose was reduced from 0.999% to 0.604%, while in the glucose urea broth the reduction was from 0.971% to 0.058%. Plainly then the urea in turn accelerated the destruction of the glucose.

A reasonable explanation of this acceleration of disintegration would appear to be that in the glucose urea broth the breakdown products of the 2 agents, glucose and urea, by maintaining the reaction nearer neutrality permit the microorganisms to act to a greater extent. To support this hypothesis the following 2 experiments were conducted:

1. Two flasks of plain urea broth of pH 8.9 were inoculated with *B. proteus*. At the end of 24 hours incubation, sufficient acid was added to one flask to reduce the pH to 6.7, reincubated for 24 hours, the reaction again adjusted to pH 6.7 and incubation continued 48 hours. It was then found that the ammonia in the corrected flask had increased 200.9 mg. as against 168.7 mg. for the uncorrected flask, which had incubated for a like period of time. This would indicate that an immoderate alkalinity restrains the breakdown of urea and supports the assumption that the greater amount of ammonia formed in glucose urea broth is due to the neutralizing action of the acid resulting from the glucose fermentation.

2. Glucose broth was distributed in 20, 50, 75, 100, 150 and 200 cc. quantities in 250 cc. Erlenmeyer flasks, one gram of finely divided $CaCO_3$ added to each, inoculated with *B. proteus* and incubated 24 hours. The insoluble $CaCO_3$ soon settles to the bottom of the flask; therefore, the larger the volume of broth in a flask of given size the further the acid must diffuse to reach the neutralizing agent. Thus if acidity was retarding glucose breakdown by *B. proteus,* there should be more glucose after 24 hours incubation in the flask containing 200 cc. of medium than in those containing lesser amounts. Such proved to be the case, the quantity of sugar being found in decreasing amounts from 0.565% in the flask with 200 cc. of medium to 0.072% in the flask with 20 cc. This favors the supposition that urea hastens the decomposition of glucose by preventing the development of acidity.

Repeated attempts to split urea in plain or glucose broth or in aqueous solution by the use of Berkefeld filtrates of cultures of *B. proteus* resulted in failure. To obtain the endo-enzyme the organ-

isms in 48-hour broth cultures of *B. proteus* were collected, washed repeatedly with saline, dehydrated with acetone and ether and then finely powdered. Testing for urease activity, by adding 0.5 gm. of the extracted organisms to 100 cc. of 0.5% urea dissolved in water, resulted in the production of 177.8 mg. of ammonia. Controls consisting of the urea solution alone and of urea solution plus 0.5 gm. of the extracted organisms, previously boiled for 10 minutes, showed no increase in ammonia. Therefore the splitting of urea, in the foregoing experiments, was brought about by an endo-urease.

Summary. Evidence is here presented that glucose and urea simultaneously present in broth media interact to accelerate the breakdown of each other by *B. proteus;* that this is in large part due to their end products maintaining the reaction at a more optimum range and that the splitting of urea is accomplished by an endocellular urease.

3963

Calcium and Phosphorus Balances of Epileptic Children Receiving a Ketogenic Diet.

MARTHA V. NELSON. (Introduced by P. C. Jeans.)

*From the Department of Pediatrics, State University of Iowa.**

Three epileptic children, 3, 7 and 9 years of age, receiving a ketogenic diet were made the subjects of a metabolic study with special reference to the calcium and phosphorus intake and output. The diets furnished approximately 1½ gm. of protein per kg. of body weight per day, and an energy value sufficient for average activity. The diet of the youngest child contained 2 gm. of potential fatty acid to each gm. of potential glucose. In the diets of the older children this ratio was 2.5:1. Each patient was excreting ketone bodies in considerable quantities and no epileptic symptoms were manifested during the period of observation. The diets were planned to include as much calcium and phosphorus as their limited carbohydrate content would allow, and were composed of cream, meat, eggs, butter and low carbohydrate vegetables and fruits. The usual metabolic procedures were employed.

In each instance the calcium and phosphorus output exceeded the intake, and the usual excess of excretion of calcium and phospho-

* Assisted by a fund from the Child Welfare Research Station, Iowa City, Ia.

rus in the stools over that in the urine was not found in these cases. There was a great increase in the urinary calcium and phosphorus, the increase of urinary calcium being proportionally greater than the increase of urinary phosphorus. Two of these children while receiving a general diet showed the average retention of calcium and phosphorus and the usual relationship between the stool and urine in the excretion of these minerals. Blood calcium and phosphorus determinations made during each period of metabolic observation were within normal limits.

In this study of 3 epileptic children the data indicate that when a ketogenic diet is employed the calcium and phosphorus output ex-

TABLE I.

Showing the calcium and phosphorus intake and output of 3 epileptic children.

No.	Intake			Output						Retention or loss				
				Urine		Stool		Total		Total		Per Kg.	
	F.A./G.	Ca.	Inorg. P.	Ca.	Inorg. P.	Ca.	Inorg. P.	Ca.	Inorg. P.	Ca.	Inorg. P.	Ca.	Inorg. P.
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	2 :1	.264	.437	.165	.323	.196	.181	.321	.504	—.097	—.067	—.008	—.005
2	2.5:1	.654	.591	.699	.895	.211	.158	.910	1.053	—.256	—.462	—.011	—.020
3	2.5:1	.688	.706	.586	.855	.233	.167	.810	1.022	—.131	—.316	—.004	—.015
2	1:2.6	1.015	1.230	.039	.525	.703	.347	.742	.872	+.273	+.358	+.012	+.011
3	1:2.6	1.184	1.435	.047	.459	.629	.559	.676	1.018	+.508	+.417	+.016	+.013

ceeds the intake, and there is a shift of the major excretion from the stool to the urine.

3964

Antipyretic Action of Magnesium Chlorid Alone and Combined With Amidopyrin.

H. G. BARBOUR AND J. E. WINTER.

From the Department of Physiology and Pharmacology, School of Medicine, University of Louisville, Kentucky.

The temperature reducing properties of magnesium salts in doses below the threshold for the well known Meltzer narcosis, have been described by J. Schütz,[1] for normal rabbits. Rabbits artificially fevered with *β-tetra-hydro-naphthylamin* also exhibited antipyresis with magnesium. The latter, however, has apparently never been investigated hitherto with regard to the reduction of fever of bacterial origin. In animals with hay-infusion fever, it is not difficult to demonstrate a marked antipyretic action with doses of magnesium salts far below the toxic range. This can be especially well shown with magnesium chlorid in rabbits previously injected with hay-infusion incubated 48 hours, at 37° C.

Healthy adult rabbits after fasting over night were injected subcutaneously with 1 cc. (per kilo) hay-infusion. A rise in temperature amounting to at least 1.5° F. was found within about 2 hours, in approximately two-thirds of the injected rabbits. The antipyretic agent was injected subcutaneously, usually 2 to 3 hours after the fever injection; at least 2 well fevered rabbits were retained for controls.

In the present work $MgCl_2$ was used in 5% solution, amidopyrin in 4% solution. When "combined" the same solutions were used, one immediately following the other; whether injected at the same or different sites was found immaterial.

The results as shown in the curves (Figs. 1 and 2) represent differences in body temperature between treated animals and untreated fever controls, the zero temperature being the temperature at the time (zero hour) of giving the magnesium chlorid.

To produce definite magnesium antipyresis in hay-infusion fever rabbits, about 200 mg.* of the chlorid per kilo are required, as il-

[1] Schütz, J., *Arch. f. exper. Pathol. u. Pharmakol.*, 1916, lxxix, 285.

* All Mg calculations are made in terms of anhydrous salts.

lustrated in Fig. 1, which shows the effect of 12 injections varying from 150 to 300 mg. per kilo. The difference between the fever control curves and those resulting from 150 mg. per kilo magnesium chlorid represents approximately the maximum variation to be expected with inadequate doses.

It has been shown in this laboratory[2] that 700 mg. per kilo represents approximately the minimal lethal dose of magnesium chlorid for rabbits.

Augmentation of Amidopyrin Antipyresis by Magnesium Chlorid.

Having shown that fevered rabbits respond to doses of magnesium chlorid amounting to less than one-third of the lethal dose, we combined magnesium with another type of antipyretic drug—amidopyrin (pyramidon). Several series of experiments were performed; typical resulting curves appear in Figs. 2 to 4. A comparison of the 3 sets of curves shown in Fig. 2, shows that a very marked antipyretic effect can be elicited by 150 to 200 mg. per kilo of amidopyrin, 100 mg., however, giving a somewhat doubtful effect.

But when to this weak amidopyrin dose is added magnesium chlorid 150 mg. per kilo, these 2 ineffective components produce in combination a profound fall in temperature. The maximum decrease obtained in an average of 9 rabbits was 3.9° F. This is greater than the effect which can be produced separately by either 200 mg. per kilo amidopyrin or 300 mg. per kilo magnesium chlorid. Hence the antipyretic combination exhibits true potentiation or synergism.

With repeated doses, we attempted a closer estimation of the therapeutic equivalent of amidopyrin in terms of its combination with magnesium chloride. In the 4 series of experiments shown in Fig. 3, 5 doses were given each rabbit at half-hourly intervals. In both amidopyrin series, the dosage was 50 mg. per kilo (every half-hour); the averages of 2 sets of 3 rabbits each, showed close agreement (maximum temperature fall about 4° F.). The combination of drugs which was compared with amidopyrin consisted of a mixture of 1 part of amidopyrin with 2 parts of magnesium chlorid. The equivalent of the amidopyrin given alone was found to lie between 50 and 75 mg. per kilo of the combination (Fig. 3).

In a further series, therefore, the 50 mg. amidopyrin dose was compared with 67.5 mg. per kilo of the combination. Injections at half-hourly intervals were performed in the 2 series which each consisted of 3 rabbits. As will be seen from Fig. 4, the combination

[2] Barbour, H. G., and Taylor, W. F., (unpublished experiments).

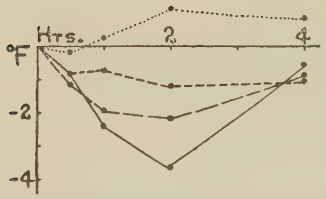

FIG. 1.

Effects of magnesium chlorid given subcutaneously, upon body temperature of fevered rabbits.

Abscissae, time after administration of antipyretic.

Ordinates, temperature changes °F, not absolute but calculated as difference from control fevered rabbits. Five fever controls.

Dosage per kilo: dotted line, 150 mg. (average of 4 rabbits); short dash line, 200 mg. (av. 2 rabbits); long dash line, 250 mg. (av. 4 rabbits); continuous line, 300 mg. (av. 2 rabbits).

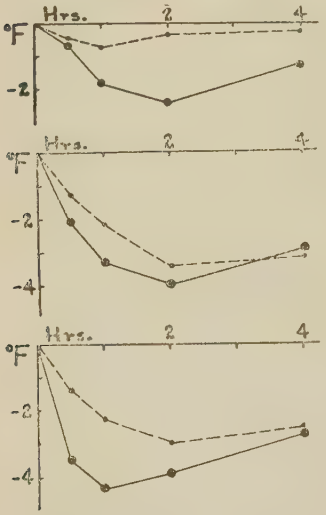

FIG. 2.

Effects of amidopyrin with and without magnesium chlorid, in fevered rabbits.

Dosage per kilo: Continuous lines, effects of amidopyrin 100 mg. plus $MgCl_2$ 150 mg. (A, 2 rabbits; B, 2 rabbits; C, 5 rabbits); broken lines, amidopyrin alone (A, 100 mg. 2 rabbits; B, 150 mg. 2 rabbits; C, 200 mg. 4 rabbits).

A, 2 fever controls; B, 1 fever control; C, 2 fever controls.

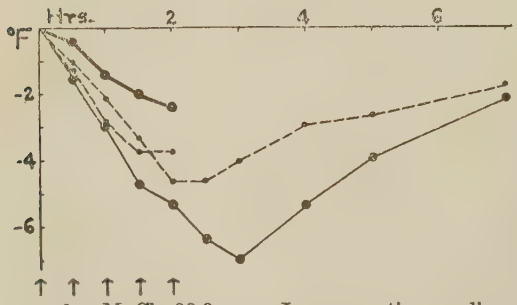

FIG. 3.

Effects of repeated doses of amidopyrin alone and combined with magnesium chlorid, in fevered rabbits.

Temperature changes absolute, no fever controls. Each curve represents the average result from 3 rabbits. Doses given half-hourly shown by arrows.

Dosage per kilo: Upper continuous line, amidopyrin 16.7 mg. plus $MgCl_2$ 33.3 mg. Lower continuous line, amidopyrin 25 mg. plus $MgCl_2$ 50 mg. Both broken lines, amidopyrin alone 50 mg.

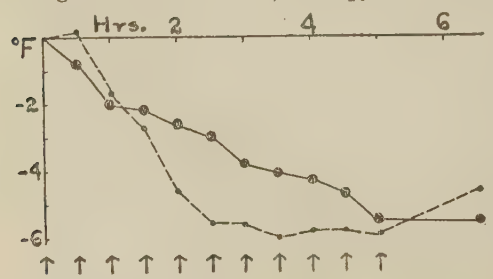

FIG. 4.

Effects of repeated doses of amidopyrin alone and combined with magnesium chlorid, in fevered rabbits.

Temperature changes absolute, no fever controls. Each curve the average from 3 rabbits. Doses given half-hourly shown by arrows.

Dosage per kilo: Continuous line, amidopyrin 22.5 mg. plus $MgCl_2$ 45 mg. Broken line, amidopyrin alone 50 mg.

maintained some advantage over amidopyrin for over an hour, that is, until the third dose was beginning to take effect, and an average fall of 2° F. had been attained. From this point on, a decidedly greater temperature fall was seen in the amidopyrin rabbits than in those given the combination.

These results are very significant inasmuch as they indicate for the magnesium-amidopyrin combination a twofold advantage over that dose of amidopyrin, causing an equal fall in 2 hours. (1) This effect was achieved more rapidly by the *combination.* (2) Further temperature reduction, signifying a *transition from the therapeutic into toxic action,* was accomplished more rapidly by the *single drug.* The latter also attained the lowest temperature.

Thus the magnesium combination exhibits both higher therapeutic efficiency and lower toxicity than does amidopyrin alone. The relative toxicity is borne out by the subsequent history of the most heavily injected rabbits (Fig. 4), for a death occurred among the 3 given amidopyrin alone, while all of those given the combination survived.

Relative Toxicity of Magnesium and Amidopyrin Alone and in Combination.

It is frequently found that the toxicity of a combination of 2 drugs differing somewhat in action, is lower than would be predicted from the known action of its components. For example, animals are likely to survive the simultaneous administration of one-half each of the fatal doses of 2 such drugs. This is readily understood from consideration of the fact that the *loci* of toxic action are more extensive. As will be seen from Table I, the minimum lethal doses have been found as follows:

$MgCl_2$	1.05 gm. per kilo.
Amidopyrin	325 mg. per kilo.
Amidopyrin + $MgCl_2$ (1:1 ratio)	500 mg. per kilo.
Amidopyrin + $MgCl_2$ (2:3 ratio)	625 mg. per kilo.

From the first 2 determinations one finds that the predicted minimum lethal doses for the 2 combinations would be 496 and 595 mg. respectively,† from which some antagonism is evident in the case of the 2:3 ratio. Thus in normal mice as well as in fevered rabbits, there is evidence in favor of a decrease in toxicity, as a result of adding to the amidopyrin quantities of magnesium chlorid which increase its efficiency.

† Prediction formula: $ab\ (A+B)/Ab+Ba$ where A parts of a drug having m.l.d.$=a$ are combined with B parts of a drug having m.l.d.$=b$.

TABLE I. *Toxicity in Normal White Mice.*

Drug*	Mg. per kilo.	Number of mice	
		Survived	Died
$MgCl_2$	1,000	2	0
	1,050	1	3
	1,100	0	4
	1,200	0	2
	1,300	0	2
	1,400	0	2
	1,500	0	2
	2,000	0	2
Amidopyrin	250	3	0
	300	7	1
	325	1	1
	350	6	4
	375	2	1
	400	7	4
	450	2	5
	500	3	4
Amidopyrin + $MgCl_2$ (2:3)	500	2	0
	562	9	1
	625	9	3
	688	8	3
Amidopyrin + $MgCl_2$ (1:1)	300	1	0
	400	2	0
	450	4	0
	500	8	2
	550	3	1
	600	0	1

*1% solutions.

Summary. 1. Magnesium chlorid proved effectively antipyretic in hay-infusion fever rabbits in doses of 200 mg. per kilo and upwards.

2. By combining doses of magnesium chlorid and amidopyrin, which separately are ineffective, marked antipyresis was produced in such rabbits.

3. The antipyretic efficiency of amidopyrin 100 mg. per kilo plus $MgCl_2$ 150 mg. per kilo was greater than the efficiency of either amidopyrin 200 mg. per kilo or $MgCl_2$ 300 mg. per kilo given separately. (Synergism.)

4. Comparing repeated doses exhibiting equivalent efficiency after 1 or 2 hours, the combination first lowered the temperature more rapidly; nevertheless the amidopyrin later produced a more rapid and extreme (toxic) effect.

5. The amidopyrin-magnesium combination (2:3) proved slightly less toxic to normal white mice than would be predicted from the known toxicity of the 2 component drugs.

Conclusion. Combinations of amidopyrin with magnesium chlorid in experimental animals exhibit antipyretic synergism, and, to a lesser extent, diminished toxicity.

3965

Influence of Magnesium Salts Upon the Toxicity and Antipyretic Action of the Salicylates.

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In the preceding paper we have shown that magnesium chlorid is antipyretic to rabbits in hay-infusion fever. Furthermore the antipyretic efficiency of amidopyrin was found considerably potentiated by combination with magnesium chlorid, although in the same combination the toxicity appears slightly decreased.

The synergistic action of magnesium combined with antipyretic drugs is further illustrated by the following experiments upon magnesium combinations with salicylates. The work will be described under 3 heads: (1) toxicity in normal mice of sodium salicylate alone and in combination with $MgCl_2$; (2) antipyretic action in fevered rabbits of sodium salicylate alone and combined with $MgCl_2$; (3) antipyretic action in fevered dogs of aspirin alone and in combination with magnesium oxide.

1. Toxicity.

In normal white mice, the m.l.d. (minimal lethal dose) of sodium salicylate was found to be about 600 mg. per kilo (Table I). Similarly, the m.l.d. of a combination of 2 parts sodium salicylate with 3 parts magnesium chlorid (anhydrous) was found to be approximately 975 mg. per kilo (Table II). Our criterion for judging the m.l.d., as will be seen, does not include an occasional fatality within that range of dosage where survival is obviously the rule.

In the previous report, it was shown that the m.l.d. for magnesium chlorid is approximately 1.05 gm. per kilo. Thus the predicted m.l.d. for the present combination with sodium salicylate would be 807.5* mg. per kilo. Inasmuch as 975 mg. were, with 1 exception, actually required, there is evidently an antagonism between the 2 drugs favorable to survival. The combination is about

* For prediction formula see other paper.

TABLE I. *Toxicity in Normal White Mice.*

Drug*	Mg. per kilo	Number of mice	
		Survived	Died
Sodium salicylate	550	5	1
	600	6	2
	650	2	2
	700	0	1
	1,000	0	2
	1,200	0	2
	1,400	0	2
	1,600	0	2
Sodium salicylate + $MgCl_2$ (2:3)	875	3	0
	900	5	1
	925	3	0
	975	0	2
	1,025	0	2
	1,050	2	1

* 1% solutions.

20% less toxic than predicted. This accords with the mutual antagonism shown between many depressant drugs when combined, and is most readily explained by the wider distribution of the *loci* of toxic action.

2. Antipyretic Action in Rabbits.

In a series of 11 rabbits, the average temperature reduction resulting from the subcutaneous injection of sodium salicylate (in 5% solution) in doses of 100 mg. per kilo, amounted to 0.7° F. in 2 hours, and 1.0° in 4 hours. These effects are illustrated in Fig. 1, where the average curve is compared with the antipyretic curves resulting from 2 different mixtures of sodium salicylate (5%) and magnesium chlorid (5%).

The above amount of sodium salicylate was combined in 8 rabbits with magnesium chloride 150 mg. per kilo (ineffective alone). The temperature fell so rapidly that the former 2-hour level of 0.7° F. was attained during the first half hour. Sodium salicylate alone had practically no effect within that period. At the end of 2 hours, the average temperature was still about half a degree below that produced by the salicylate alone, but by the fourth hour, the magnesium effect wearing off, the temperature had begun to return to normal so that the position of the 2 curves nearly coincided.

Even when only 50 mg. per kilo of sodium salicylate were given with magnesium chlorid 150 mg. per kilo rapid reduction in temperature was noted. This combination was found more efficient in 13 rabbits, than 100 mg. per kilo sodium salicylate alone, for a period of over one and one-half hours. By the fourth hour, however, the temperature had returned practically to the original febrile level.

FIG. 1. Effects in fevered rabbits of subcutaneous administration of sodium salicylate alone and with magnesium chlorid, upon body temperature.

Abscissae, time after administration of antipyretic.

Ordinates, temperature changes °F., not absolute but calculated as difference from control fevered rabbits. Seventeen fever controls.

Dosage per kilo: Broken line, Na salicylate 100 mg. (average of 11 rabbits). Light continuous line, Na salicylate 50 mg. plus $MgCl_2$ 150 mg. (av. 13 rabbits). Heavy continuous line, Na Salicylate 100 mg. plus $MgCl_2$ 150 mg. (av. 8 rabbits).

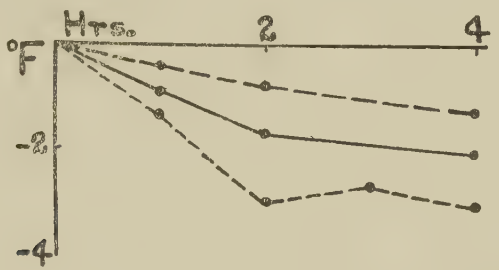

FIG. 2. Effects in fevered dogs of oral administration of aspirin alone and with magnesium chlorid. No fever controls. The temperature decreases are absolute rather than relative.

Dosage per kilo: Upper broken line, aspirin 100 mg. (1 dog). Lower broken line, aspirin 200 mg. (av. 2 dogs). Continuous line, aspirin 100 mg. plus $MgCl_2$ 150 mg. (av. 4 dogs).

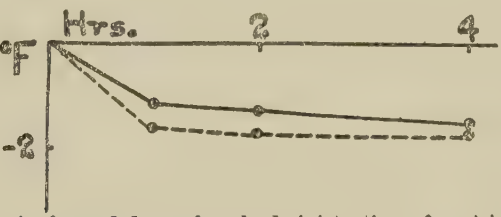

FIG. 3. Effects in fevered dogs of oral administration of aspirin alone and with magnesium oxid. Four fever controls.

Dosage per kilo: Broken line, aspirin 150 mg. (av. 4 dogs). Continuous line, aspirin 100 mg. plus MgO 100 mg. (av. 5 dogs).

FIG. 4. Effects in fevered dogs of oral administration of magnesium oxid. One fever control.

Dosage per kilo: Dotted line, MgO 75 mg. (1 dog). Short dash line, MgO 150 mg. (1 dog). Long dash line, MgO 300 mg. (1 dog).

The slow, long-continued temperature fall produced by salicylate is thus shown to be modified to a more rapid though briefer effect when magnesium chlorid is added. It is significant that amounts of magnesium chlorid below the antipyretic threshold are sufficient to cause such a modification even when the salicylate dose is cut in half. Thus marked synergism outlasts the first hour. The crossing of the curves after a certain time is analogous to that noted with amidopyrin-magnesium antipyresis and definitely indicates that magnesium salts potentiate the early effects of antipyretic drugs, even when intense or prolonged action tends to be inhibited.

3. Antipyretic Action of Aspirin and Magnesium by Mouth in Dogs.

Aspirin administered by mouth† to fasting, fevered dogs in doses of 100 and 200 mg. per kilo, gave respective decreases in temperature of 1.3° (1 dog) and 3.1° F. (2 dogs) at the end of 4 hours (Fig. 2). The curve of antipyresis resulting from a combination of aspirin 100 mg. per kilo plus magnesium chlorid 150 mg. per kilo (4 dogs), occupies an almost exactly intermediate position. The combination thus accomplished the same result as would require about 150 mg. per kilo of aspirin alone.

Further experimental series were performed, using magnesium oxide in heavy suspension instead of the chlorid. In substituting magnesium oxide for magnesium chlorid, the dose used was reduced by one-third, giving a slightly higher molecular concentration of magnesium. As seen in Fig. 3, this combination (100 mg. of each drug) was not as effective as 150 mg. per kilo of aspirin alone, although presumably better than would be obtained by 100 mg. per kilo. The effect of the combination for the first 2 hours was slightly better than that produced by 150 mg. per kilo magnesium oxid alone, and did not show the remission seen 4 hours after the latter. (See Fig. 4, which shows the effects of 3 different doses of magnesium oxide.)

The weaker action of magnesium oxide as compared with the chlorid may be ascribed to slower absorption.

Conclusions. 1. Magnesium salts in mice appear to reduce the toxicity of salicylates (protective antagonism).

2. Magnesium augments the antipyretic action of sodium salicylate and of aspirin. When given subcutaneously with salicylate to fevered rabbits, the earlier stages of antipyresis are characterized by marked synergism.

† Suspended in about 200 cc. H_2O with or without acacia.

3966

Antagonism of Nicotine Action by Cocaine.

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In an attempt to analyze the action of cocaine on excised smooth muscles, it was discovered that treatment of a segment of mammalian intestine with nicotine following treatment with cocaine, failed to elicit the usual nicotine response. As is well known, the action of nicotine on excised intestinal strips is one of augmentation, followed by depression, with gradual recovery. Some segments, however, are only depressed by nicotine. The experiment was tried with Magnus preparations of small intestine and colon of the cat, dog, rabbit and guinea pig, Tyrode solution being used as the bath. For every segment of intestine treated with cocaine before the addition of nicotine to the bath, there was used an adjacent control segment, treated with nicotine alone. Out of nearly a score of trials, only once did cocaine fail to prevent nicotine action. Contact of cocaine with the tissue for a period of 3 minutes was sufficient to prevent nicotine action, even if the cocaine solution were replaced with fresh Tyrode solution before administration of the nicotine. The concentration of nicotine used was from 1:250,000 to 1:50:000 and that of cocaine, 1:10:000.

Cocaine failed to prevent depression of intestinal segments by atropine.

In a previous report on cocaine[1] it was suggested that this alkaloid acted mainly on smooth muscle. The antagonism of nicotine by cocaine and the failure of cocaine to antagonize atropine, would indicate that cocaine affects the ganglia, or at least some portion of the enteric nervous system, as well as the smooth muscle.

[1] Thienes, C. H., *J. Pharm. Exp. Ther.*, 1928, in press.

3967

Phytopharmacology: Effect of Ricin, Capsicum and Cantharides on Growth of Lupinus Albus.

DAVID I. MACHT.

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The author has called attention in numerous papers to the fact that living plant protoplasm may be a useful pharmacological test object and that indeed such phytopharmacological preparations may respond with greater sensitiveness to certain poisons than do living animal tissues.[1] He has also shown that plant pharmacological preparations may be especially sensitive to the effects of poisons of animal origin, possibly because such products are more foreign to plants than are the metabolic products of plant life.[2] In order to test this hypothesis the present experiments were undertaken and the results obtained are unequivocal. The author selected for study the effect on the growth of seedlings of *Lupinus Albus* 2 substances known as extremely poisonous, one of plant origin and the other of animal origin, namely, *Ricin* and *Cantharadin.* Ricin is well known to pharmacologists and toxicologists as one of the most deadly poisons, a few milligrams sufficing to produce death of a large animal. This substance sometimes spoken of as a toxalbumin is obtained from from the castor oil bean. *Cantharadin* is a crystalline body also of a very poisonous nature obtained from the Spanish fly *(Cantharis Vesicatoria).* Very dilute solutions of these 2 drugs were made and the effects of such solutions on the growth of *Lupinus Albus* seedlings were studied according to the methods already described. To prepare a solution or suspension of *Ricin,* in some experiments the drug was rubbed up in a mortar with cold saline, in other experiments a suspension or solution of the same was prepared by heating this substance in a large volume of distilled water. A solution of *Cantharidin* was prepared in a similar way. The results obtained are shown in the subjoined table. It will be noted that *Ricin* produced but very little inhibition in the growth of the roots and indeed was less toxic even than normal blood serum. A suspension made with cold water by trituration in a mortar produced no toxic effect at all. A solution prepared by heating *Ricin* with water for subsequent dilutions with Shive solution gave a growth

[1] Macht, D. I., and Livingston, M. B., *J. Gen. Physiol.*, 1922, iv, 573.

[2] Macht, D. I., *J. Am. Med. Assn.*, 1927, lxxxix, 753.

of 91%. The action of *Cantharidin* even after great dilution was very different. Solutions of 1:50,000 practically killed the plants, giving an index of growth of only about 3%. The action of *Cantharides* in the form of tincture was also compared with the tincture of another drug exhibiting somewhat similar irritant properties in animals, namely, *Tincture of Capsicum.* Here again a marked difference was noted between *Capsicum,* the drug of plant origin, and *Tincture of Cantharides,* the drug of animal origin. Taking into consideration the alcohol content of each tincture and making proper controls for the same it was found that tincture of *Cantharides* was at least twice as toxic for the growth of *Lupinus Albus* as tincture of *Capsicum.*

It is, therefore, evident that so far as the above drugs are concerned, the ones of animal origin are very much more poisonous for plants than the 2 of plant origin. This statement, however, does not hold good for all drugs or chemicals obtained from animals, especially those which are normal physiological products of animal metabolism. The author has found that neither Epinephrin, nor Pituitary Extracts, nor Histamine, nor Insulin are very toxic for the growth of *Lupinus Albus* seedlings.

TABLE I.

Drug	Dosage	Phytotoxic Index
Ricin	1:50,000 Prepared cold	100%
Cantharidin	1:50,000	4%
Ricin	1:25,000 Prepared by heating	91%
Cantharidin	1:50,000	3%
Alcohol 95%	1% control	70%
Tinctura Capsici	1% solution	27%
Tinctura Cantharidis	1% solution	54%

3968

A Direct Method for Making Total White Blood Counts on Avian Blood.

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During the course of a standardization of the blood of fowls, undertaken as a part of a series of studies upon acid fast infections in various species of animals, it was found that no direct method existed for making total white blood counts upon avian blood. The methods commonly employed for mammalian blood, which embody the principle of destruction of the red cells in order that the white cells may thereby be counted without confusion, are not satisfactory for avian blood. This is because the circulating red blood cells in the bird are nucleated and, while acetic acid solutions hemolyze the red cells, they will not destroy their nuclei; the latter are thus liberated and are almost indistinguishable from small lymphocytes.

Because of this difficulty, various indirect methods based upon the ratio of red blood cells to white cells in the same smear were devised for making estimations of the total white cell counts of the bird. The reports in the literature for the blood of normal fowls when such methods were employed are quite divergent. Among the most careful studies that have been reported are those of Schmeisser[1] and of Warthin.[2] The former determined the ratio of red blood cells to white blood cells from smears stained with Wilson's stain. He found the ratio to vary between 1 to 40 and 1 to 150 with an average of 1 to 50. Using these ratios he calculated the total white blood counts and found they varied between 20,000 and 80,000 cells per cmm. Warthin, with similar technique, found the ratios between red cells and white cells to vary between 1 to 102 and 1 to 225, and the total white counts to vary between 12,000 and 29,000 cells per cmm.

In view of such divergent findings from the use of the indirect method, it was felt advisable to develop a direct method for making white blood counts upon chicken's blood. Inasmuch as the nuclei of the red blood cells can not be destroyed without destruction of the white blood cells it seemed indicated to devise a method which

* This work has been assisted by a grant from the Henry Strong Denison Medical Foundation.

[1] Schmeisser, H. C., *Johns Hopkins Hosp. Reports*, 1916, xvii, 551.

[2] Warthin, A. S., *J. Infect. Dis.*, 1907, iv, 369.

would leave the erythrocytes intact, and at the same time would stain the leucocytes without also staining the red cells. The ease with which neutral red is taken up by living white cells and the success of Gardner[3] and of Cash[4] in fixing the neutral staining granules of cells so that they may be studied in fixed material suggested a possible modification of these methods to meet the requisites of total white counts upon the fowl.

For this purpose two diluting fluids were employed. Solution I contained neutral red (1:5000) made up in Lock's solution and adjusted to a pH of 7.4. Solution II contained 12% formalin, also made up in Lock's solution and adjusted to a pH of 7.4. The procedure employed for diluting the blood with these solutions was as follows: The blood was drawn up to the 0.5 mark in a standard red blood pipette. The solution of neutral red (which was kept at a temperature of 39° C.) was then drawn into the pipette until the bulb was about one half full, and the pipette was shaken for 15 seconds. Following this, the pipette was filled to the 101 mark with the formalin solution, and shaking was continued for 2 to 3 minutes after which the counting chamber was filled. It was found that every white cell had taken up sufficient neutral red to make identification from the red cells, which had taken up no neutral red, very easy under a 4 mm. lens.

The accuracy of the method was determined in 2 ways. First, repeated counts were made upon the same chicken at different hours on the same day. Secondly, the method was employed simultaneously with the use of Turk's solution for counts upon human blood. Table I gives these comparison counts.

TABLE I.
Control counts of total white blood cells.

Chicken No. 241		Human Blood	
Hour	Neutral Red and Formalin Sol.	Turk's Sol.	Neutral Red and Formalin Sol.
1:00	26,800	6,000	6,500
1:30	26,000	5,100	4,900
2:00	29,600	5,100	4,950
2:30	28,000	5,650	5,250
3:00	29,600	7,100	6,650
		5,750	5,925
		5,450	5,250
		6,200	6,250
		6,350	6,100
		5,600	5,800

[3] Gardner, L. U., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 646.

[4] Cash, J. R., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 193.

It will be seen from the table that the variations in the different counts are not greater than would normally be expected. Counts made at the same time either with different methods or as duplicates with the same method agree within the usual limits. Counts made from hour to hour show variations which are in accord with those which Sabin, Cunningham, Doan and Kindwall[5] have shown to be normal. The greatly divergent variations reported from the use of the indirect methods are, however, not present, and it is permissible to conclude that this method offers increased accuracy over those previously employed in making total white counts of avian blood in which the nuclei of red blood cells are invariably present.

3969

Mechanism of Allergy in Tuberculosis.

ARNOLD R. RICH AND MARGARET R. LEWIS. (Introduced by L. E. Holt, Jr.)

From the Department of Pathology, The Johns Hopkins University, and the Department of Embryology, The Carnegie Institute of Washington.

These experiments are part of an investigation into the mechanism of allergy in tuberculosis and its relation to immunity. This report is concerned with the problem of individual cell sensitiveness in the allergic animal.

The tuberculin reaction and the allergic lesions occurring in the body during the progress of infection are generally regarded as being, most probably, the result of an antigen-antibody reaction, in which bacillary products react with an antibody formed during the course of the infection. Rich and McCordock have been unsuccessful in attempts to demonstrate satisfactorily, *in vivo,* the presence of such an antibody in the plasma of allergic animals, and this has been the experience of others who have recently made similar attempts. The surmise has therefore arisen that if an antibody-antigen reaction is indeed responsible for the allergic inflammation and necrosis, this reaction probably takes place in or on the cells themselves, to which the antibody may be bound.

It has never been proven, however, that the isolated cells of the allergic body are really changed in a manner which renders them more sensitive than normal cells to the effects of the products of the tubercle bacillus. Studies *in vivo* are complicated by the presence

[5] Sabin, F. R., Cunningham, R. S., Doan, C. A., Kindwall, J. A., *Johns Hopkins Hosp. Bull.*, 1925, xxxvii, 14.

of the body fluids, by circulatory changes, by uncontrollable variations in antigen concentration which result from the local fixation of the antigen in allergic animals, and by other factors. The purpose of the present experiments was, therefore, to test the sensitiveness of isolated cells to tuberculin *in vitro,* in a manner which might give us information as to whether or not the individual cells of the allergic body become altered in such a way that, regardless of the medium by which they are surrounded, they will be damaged by a given amount of tuberculin more readily than normal cells. To this end we have used the method of tissue culture. We have exposed the washed cells of normal and of allergic guinea pigs to measured amounts of tuberculin, and we have, in each experiment, combined the cells and plasmas in the various ways which might throw light upon the question as to whether a humoral factor is involved in the allergic reaction.

Our experiments, which include hundreds of cultures of spleen and of white blood cells, show in the clearest manner that the cells of an allergic animal are much more sensitive to the effects of tuberculin than are those of a normal animal. They are killed by amounts of tuberculin in which normal cells grow freely. The sensitiveness of the washed cells of each allergic animal was exhibited in the same degree, regardless of whether they were exposed to tuberculin in the plasma of normal or of allergic animals. Tuberculin in allergic plasma had no more effect upon normal cells than had tuberculin in normal plasma. The table summarizes part of a typical experiment:

TABLE I.

Washed cells from	Grown in plasma from	Added to plasma at start	Migration and growth	Condition of cells
Normal animal	Normal animal	1:60 tuberculin	Very good	Very good during 4 days
Normal animal	Allergic animal	1:60 tuberculin	Very good	Very good during 4 days
Normal animal	Normal animal	1:60 concentrated glycerine broth control	Very good	Very good during 4 days
Normal animal	Allergic animal	1:60 concentrated glycerine broth control	Very good	Very good during 4 days
Allergic animal	Normal animal	1:60 concentrated glycerine broth control	Very good	Very good during 4 days
Allergic animal	Allergic animal	1:60 concentrated glycerine broth control	Very good	Very good during 4 days
Allergic animal	Normal animal	1:60 tuberculin	Very slight	Most cells dead within 48 hrs.
Allergic animal	Allergic animal	1:60 tuberculin	Very slight	Most cells dead within 48 hrs.

We are at present examining the Arthus phenomenon in a similar manner, and also the reaction of normal and allergic cells to the intact tubercle bacillus.

Conclusion. Cellular injury and necrosis associated with allergy in tuberculosis result from a change in the individual fixed tissue and blood cells, which renders them more sensitive to the products of the tubercle bacillus. There are no humoral factors necessary for the production of the injurious local effects of allergy in tuberculosis.

3970

Rate of Reflex Conduction in a Cataleptic Patient.

LEE EDWARD TRAVIS. (Introduced by W. W. Tuttle.)

From the Psychopathic Hospital, State University of Iowa.

A previous study of normal subjects has well established the fact that in the case of the knee-jerk the average length of time elapsing between the instant of stimulation of the patellar tendon and the appearance of action currents in the executant muscle is .0197 seconds.[1] This average is based upon records obtained from 87 individuals who presented reflex times ranging from .0114 to .0268 seconds. Reflex conduction rate is relatively constant for the same individual under normal conditions. However it was thought that certain mental and physical abnormalities might affect it and with this thought in mind a study was made of a case diagnosed as toxic-infective-exhaustive psychosis. When the first records were obtained the patient displayed catalepsy, *cerea flexibilitas,* muteness, stupor, and absolute disorientation. The findings revealed her reflex time to be .0059 seconds. Four days after these records were obtained she showed marked improvement—the catalepsy and *cerea flexibilitas* had practically disappeared, and she was talking and enjoying herself with other patients. Records now revealed the fact that her reflex time was .0180 seconds. Twenty-five days later when she was discharged as "physically improved and mentally recovered" her reflex time was .0185 seconds. Thus it appears that the catatonia here was accompanied by a markedly reduced patellar tendon reflex time, and that as the catatonic condition cleared up the reflex time approached the average for normal indi-

[1] Travis, Lee Edward, *Science,* Jan. 13, 1928.

viduals. An explanation of this increase in reflex conduction rate during catalepsy is difficult to make. It may be due to increased facilitation of transmission of the nerve impulse, to blocking out of parts of the arc ordinarily traversed by the nerve impulse, or to more obscure and involved alterations of neural, neuromuscular, or muscular factors.

Another study has indicated that the Betz cells in the precentral cortex may constitute part of the arc which subserves the knee-jerk.[2] Studies which we are now carrying on relative to the effects of emotion, stuttering, physical disease, and alcohol upon reflex time show still better that the cortical arcs are involved in the patellar tendon reflex. Thus it would seem that in catalepsy the reduced reflex time is due to blocking out of parts of the arc which ordinarily function in the knee-jerk response.

3971

The Colonic-Jejunal Reflex in the Dog.*

ARTHUR H. STEINHAUS, CHARLES B. STANWOOD, JOHN AND FLETCHER SLATER AND GEORGE SCHEUCHENPFLUG.
(Introduced by A. J. Carlson.)

From the Physiological Laboratory of the University of Chicago and The Y. M. C. A. College Laboratory for Physiologic Research in Physical Education.

In a study of the influence of treadmill running on jejunal Thiry fistula motility in the dog, variable results were secured. Thus at times there was complete inhibition of motility and reduced tone during the running, whereas at other times the motility was little modified. Chance observations, on several occasions, associated balking, attempts to sit down, and defecations while running with reduced jejunal tone and motility. The plausibility of the notion that all of these "misbehaviors" might be expressions of the defecatory reflex and thus have an ultimate seat in colonic activity and that this might help to untangle the mechanism by which exercise operates on the jejunum, led us to investigate more specifically into the influence of colonic stimulation on the jejunal motility and tone. Two approaches appeared obvious: 1. To observe the jejunal mo-

[2] Travis, L. E., Tuttle, W. W., and Hunter, T. A., *Am. J. Physiol.*, 1927, lxxxi, 670.

* A grant from the Laura Spelman Rockefeller Memorial to The Y. M. C. A. College has aided in the conduct of this study.

tility when the colon is expressly stimulated as by distension. 2. To observe simultaneously the spontaneous activity of jejunum and colon at rest and during exercise to determine the interactions resulting from peristalsis, etc., originating in either.

The latter approach, which appears to lead to disclosure of the more natural conditions, is a tedious one and our data is accumulating slowly. The first approach has led us into a field where King[1] has already done careful work. Pearcy and Van Liere[2] and Pearcy and Weaver,[3] working on animals under anesthesia or after decerebration, have also reported several relevant findings.

The observations here reported were made on 3 dogs with completely healed Thiry fistulas. The fistula balloon, essentially as described by Ivy and Vloedman,[4] is connected by rubber tubing with a specially designed water manometer. This manometer (Fig. 1) embodies the principle of the hydraulic press and serves to reduce the amplitude of the writing arm excursions. The instrument has been standardized so that by use of appropriate factors the actual air pressure in the balloon measured in cm. of water is readily calculated; also the work done in a unit of time is quickly figured. The amount of air injected is always the same (16 cc.) and the dogs always stand throughout the tests.

The colon and rectum were stimulated first by the passage of a glass tube and by a discharge of air into the colon, and later by inflation of a special rectal balloon which we devised and later found described by Pearcy and Van Liere[2] in their work on the colonic gastric reflex.

Our findings may be summarized as follows:

1. Mechanical irritation, as by passing or twisting a rod in the rectum or lower colon causes jejunal inhibition.

2. Stimulation by distension or stretching of the rectum or lower colon also causes jejunal inhibition.

3. It appears that mechanical irritiation is most effective in the rectal region and distension proves most effective at a distance of 20 to 30 cm. from the anus, where mechanical irritation is with less effect. A distension pressure effective at 30 cm. from the anus may be without effect at 15 cm. from the anus.

4. All of the reflexes are more easily elicited after 24 hours of starvation than shortly after eating.

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[1] King, C. E., *Am. J. Physiol.*, 1924, lxx, 183.

[2] Pearcy, J. F., and Van Liere, E. J., *Am. J. Physiol.*, 1926, lxxviii, 64.

[3] Pearcy, J. F., and Weaver, M. M., *Am. J. Physiol.*, 1927, lxxxii, 47.

[4] Ivy, A. C., and Vloedman, C. A., *Am. J. Physiol.*, 1923, lxvi, 141.

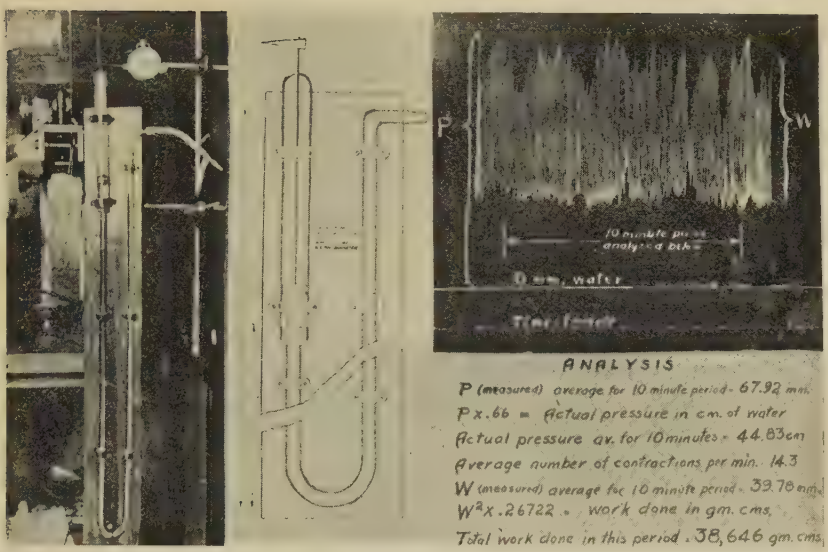

FIG. 1. The special water manometer designed to reduce the amplitude of the contraction record and a sample tracing with formulae for calculating actual pressure and work done. A manometer of this type permits of a more isotonic contraction of the gut musculature than does the average instrument of uniform diameter.

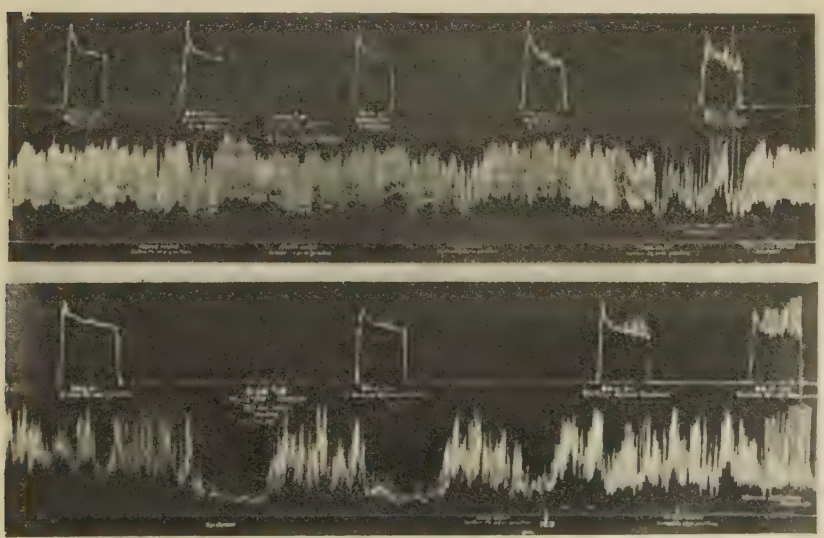

FIG. 2. Simultaneous record of jejunal activity and colonic distension stimuli. The upper line in each tracing is the record of a mercury manometer connected with the colonic balloon. The average pressure is 90 mm. Hg. The lower line is written by the special water manometer (Fig. 1) and records an average pressure around 400 mm. H_2O and the highest about 500 mm. H_2O.

The upper tracing, taken 6 hours after eating, shows exaggerated jejunal motility in place of inhibition following colonic distension (compare with Fig. 3 from same dog before eating). With distension applied 30 cm. from anus there is some inhibition. Presence of the uninflated balloon (mechanical stimulus due to moving balloon) 5 cm. from anus causes jejunal inhibition marked by defecatory contractions.

In *the lower tracing* two apparently spontaneous inhibition periods of identical configuration are recorded and a third which began in typical fashion was instantly terminated when the dog was fed at point marked by arrow.

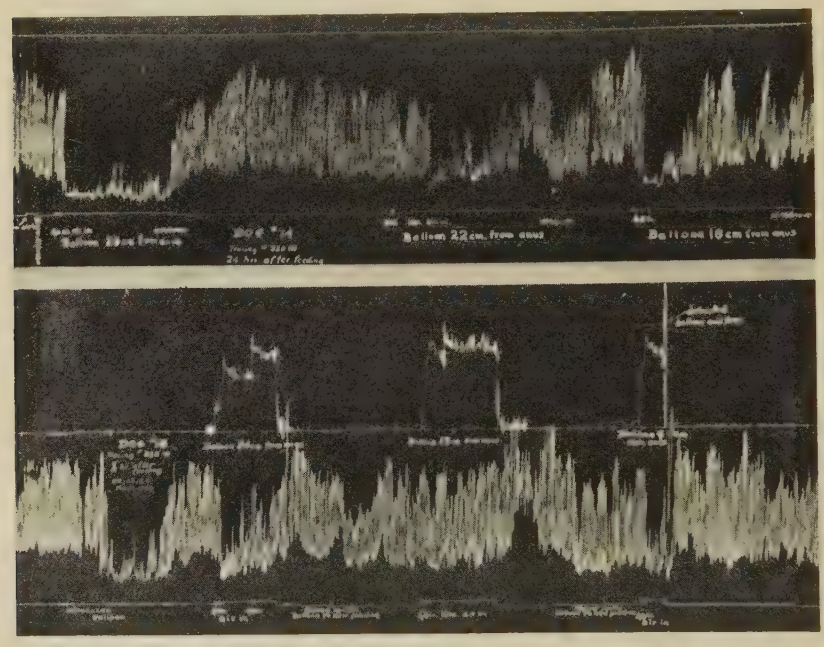

FIG. 3. Tracings from a dog (same as in Fig. 2 upper) taken immediately before and after feeding.

In *the upper tracing* no record is made of the colonic pressure. It was essentially equal to that in the lower tracing, averaging 90 mm. Hg. It shows the graduated inhibition response which is greatest when the distension is high in the colon (complete at 30 cm. from anus) and less when it is at lower levels.

The *lower tracing* (after feeding) shows exaggerated motility without inhibition following distension at the same level which before feeding showed marked inhibition. The manometer here recorded a pressure close to 600 mm. H_2O produced by jejunal contractions.

5. In the starving animal there are frequent spontaneous periods of jejunal inhibition. Because such a spontaneous period may be immediately terminated by feeding the animal suggests that hunger contractions may be responsible. We intend to pursue this point farther.

6. Following a period of inhibition induced by colonic stimulation the jejunum shows a period of exaggerated activity lasting as long as 10 minutes.

7. A distension stimulus insufficient to cause jejunal inhibition may cause increased motility even during the time of its application in the colon. There is some indication of this in the work of King. The view that the post-stimulation hypermotility is also the positive result of milder stimulation bringing into action an accelerator system rather than a "rebound" or "after-effect" phenomenon, is made more tenable and deserves further consideration.

8. Distension of the rectum results in contractions at this point. We have, however, been unable to verify the claim of Pearcy and Van Liere that contractions arise wherever the gut is distended. In fact, 15 to 30 cm. from the anus distension was followed by progressive relaxation. Defectory contractions were observed only when the balloon was close to the anus.

9. The jejunal contraction rate is remarkably constant varying very little from an average of 15.5 contractions per minute. This is not influenced in any way by changes in the respiratory rate.

Numbers 1, 2, and 6, corroborate the findings of others whose work is here cited.

3972

Dietary Requirements for Fertility and Lactation.* XVI. Potency of "Vitavose"† versus Dehydrated Yeast in Vitamin B.

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Yeast and wheat embryo are the 2 most potent sources of vitamin B. I have demonstrated[1] that, while a cold 75% ethyl alcoholic extract of 6.5 gm. whole wheat embryo per animal per day furnishes enough vitamin B for excellent growth, it is necessary to supply the same alcoholic extract of at least 22.4 gm. per lactating rat daily to furnish enough vitamin B for normal lactation.

* Research Paper No. 61, Journal Series, University of Arkansas.

† "Vitavose" is a commercial wheat germ extract manufactured by E. R. Squibbs & Sons, New York. It is used extensively by some nutritional laboratories as a source of vitamin B for growth.

[1] Sure, B., *J. Biol. Chem.*, 1927, lxxiv, 55.

Recently I have perfected a quantitative biological method for the study of vitamin B requirements for lactation.[2] The technique consists of administering to lactating mothers *(Mus norvegicus albinus)* rearing litters of 6 young at the first plateau curve of the nurslings (showing depletion of vitamin B reserves of the mothers) quantitatively graduated amounts of dehydrated yeast, or concentrated extracts therefrom, separately from the ration. By such technique it became apparent that is was necessary to furnish 1500 mg. of Harris yeast‡ daily to the lactating mothers to enable them successfully to wean their litters. This amount of yeast dosage, we have determined, is approximately 3 times that required for optimum growth of the adult and non-lactating rat.

We have had occasion[2] to examine by our rather delicate biological method the potency of a dehydrated baker's yeast.§ Twenty-three lactating mothers with litters of 138 young were employed to assay that brand of dehydrated yeast. Sixteen mothers successfully weaned 88 out of 96 young on a daily dosage of 1500 mg., or a lactation efficiency index of 91.6%; and 7 mothers weaned 41 out of 42 young on a daily dosage of only 1200 mg., or a lactation efficiency index of 97.6%. Our method discloses that, in so far as lactation requirement is concerned, the Harris yeast, claimed to be a concentrated extract from brewer's yeast, has no greater biological value than the "Federal" brand of dehydrated baker's yeast.

In this communication our results on the biological value of "Vitavose" (which is used by a number of investigators apparently successfully for vitamin B growth requirements of the dog)[3] as a source of vitamin B for lactation are briefly summarized, the findings of which may be of interest to other workers engaged in studies of dietary requirements for milk secretion.

We were very much surprised to find, in view of the manufacturer's claim that the vitamin B content of "Vitavose" is equal to that of dry brewer's yeast, that as much as 2500 mg. of that product administered daily to nursing mothers at the critical point of the depletion of her vitamin B reserves are absolutely ineffectual for the physiological function of lactation. In addition to the curative method we have also employed the preventive method of administering "Vitavose" to the lactating mothers from the date of the birth

[2] Sure, B., *J. Biol. Chem.*, 1928, lxxvi, 673.

‡ The "Harris yeast" is a commercial extract from brewer's yeast.

§ The brand of that dehydrated yeast is "Federal," furnished by the Eli Lilly & Co., Indianapolis, Ind.

[3] Williams, Geo. A., *Am. J. Physiol.*, 1927, lxxxiii, 3.

of the litters but without any success. We have employed 18 mothers which were allowed a total of 108 young to be reared, the daily dosage allowed the mothers was 1500 to 2500 mg. and the infant mortality was 100%. When at the critical point in lactation a daily dosage of 2500 mg. of "Vitavose," which proved a failure as a source of vitamin B for milk secretion, was replaced by a daily allowance of 1500 to 1800 mg. of dehydrated brewer's yeast (furnished by the Schlitz Beverage Co., 7 years old and which might have even deteriorated in vitamin potency due to aging) lactation was not only improved but the majority of the young were successfully weaned.

"Vitavose" compared with dehydrated brewer's yeast or dehydrated baker's yeast proved to be a complete failure as the only source of vitamin B for lactation.

3973

A New Means of Control of Action of Ciliated Epithelium. Effect of Moisture.

JAMES F. McDONALD, C. E. LEISURE AND E. E. LENNEMAN.
(Introduced by Victor E. Levine.)

From the Department of Physiology, School of Medicine, Creighton University, Omaha, Nebraska.

It has been proved by the authors that ciliary movement, though initiated automatically, is regulated, like movement of other types of visceral contractile tissue of higher organisms (vertebrate), by sympathetic and parasympathetic nervous acceleration and inhibition, and by sympatho- and parasympatho-mimetic types of chemicals. We here present another factor of control of the rate of activity of this tissue, not described heretofore, namely, moisture. We have found during the last 2 years that the propulsive power of ciliated epithelium is absolutely dependent upon the presence of a surface film of fluid, since this power is completely paralyzed by drying. The method for determining ciliary speed rate was the same as that formerly reported.[1] The average time in seconds required for a fine, light particle upon the mucus membrane of the palate and pharynx of the frog to cross the field of a binocular microscope was

[1] McDonald, James F., Leisure, C. E., and Lenneman, E. E., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 968.

recorded. The movement, or lack of movement, during and after various degrees of drying of the mucus membrane was recorded. Drying was done by blowing air gently from a rubber bulb upon the surface. We have also used mammals in similar experiments.

In every case evaporation of the surface film of moisture slowed and finally stopped completely its propulsive action, when drying was maintained. The particle remained at a stand-still upon the mucosa as long as the ciliated surface lacked a film of fluid. When moisture (physiological saline, Ringer's solution, or tap water) was again added after incomplete drying the ciliary propulsive action was resumed but at a slower rate. Ciliary activity was not resumed upon the addition of moisture after complete drying.

We also confirmed repeatedly the principle, noted by Kraft,[2] Gray,[3] and others, that the rate of ciliary activity is profoundly influenced by temperature, since on cold days one to several minutes was required for the particle to cross the microscopic field; whereas in hot weather its speed was rapid.

It seems that the principle of the utter dependence of the propulsive power of cilia upon moisture should be highly important in relation to the respiratory function of higher organisms including man, because the brunt of the task of filtering a pure stream of oxygen for the blood and tissues out of an abundantly dust- and bacteria-polluted inspiratory air stream is normally borne by the sheet of ciliated epithelial tissue lining the respiratory passages. In fact, this is the only visceral system in extensive, intimate, and continuous contact with the outside environment.

We have evidence from studying the nasal cavities and accessory sinuses of the mammal that these cavities may form an auxiliary irrigation plant for delivery of moisture from their glands, via ciliary propulsion, to the nose. The increased lachrymal secretion from nasal irritation seems to have a similar function.

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[2] Kraft, H., *Arch. ges. Physiol.*, xlvii, 196.

[3] Gray, J., *Royal Soc. B.*, Series xcv, 6.

3974

A Body Plethysmograph for the Study of Respiratory Movements in Human Beings.

CARL A. L. BINGER AND JOHN S. DAVIS, JR.

From the Hospital of the Rockefeller Institute, New York.

The difficulty of obtaining satisfactory records of the depth and rate of respiration in untrained human subjects is known to all who have attempted to make these measurements. Even training, though it results in regular, quiet breathing, undoubtedly alters the normal, unrecorded respiratory movements. These difficulties are much magnified when dealing with patients who are suffering from embarrassed breathing, such as occurs in pneumonia. The presence of a mask or mouthpiece may alter the depth and rate of breathing profoundly. More important may be the changes brought on by the subject's consciousness of the fact that his respirations are being studied.

To circumvent these sources of error we have constructed a body plethysmograph, by means of which it is possible to record the respiratory movements of a patient sick with pneumonia without further embarrassment to his breathing, and, indeed, without his knowledge that his respirations are being observed. The apparatus consists of an air-tight box in which the subject's body is enclosed, his head projecting out of it through a rubber dam, which makes a tight seal about the neck.

The plethysmograph is not in principle different from ones used in animal experimentation. Since the adaptation of such an apparatus to the human subject required certain changes to meet the needs of comfort and convenience, particularly of temperature regulation, we have thought it worth while to publish a more or less detailed description of it.

The subject is transferred from his bed on to a hair mattress 152 cm. long by 79 cm. wide and 8 cm. thick. This mattress is countersunk into a solid platform which forms the base of the plethysmograph. A pillow is placed under the subject's knees. His neck lies in a semicircular yoke, and his head on an adjustable head rest. A rubber dam having at its center a circular hole approximately 6 cm. in diameter is pulled over the patient's head in such a manner that the edges of the hole fit snugly to the skin of the neck. The rubber dam is fortified about its periphery by a frame of heavy rubber sheathing 28 cm. square to which is has previously been cemented.

When the patient is accustomed to his new position and is quite comfortable, the cover of the plethysmograph is put into place. This consists of a tunnel-shaped aluminum structure supported and made rigid by iron arches and flanged by an iron frame 158 cm. by 87 cm. in dimensions. The radius of the arc of the tunnel is 47 cm. To fasten the aluminum tunnel securely to the oak platform, the flat surface of the iron frame is clamped by a series of eccentric clamps on to a gasket of thick walled pressure tubing which is countersunk into the oak platform. A semicircular opening at the head end of the tunnel coincides with the previously mentioned semicircular yoke to make a circular orifice through which the patient's neck projects. The diameter of the orifice is 17 cm. This is closed by the rubber dam, held in place by a hinged metal hoop which presses the rubber

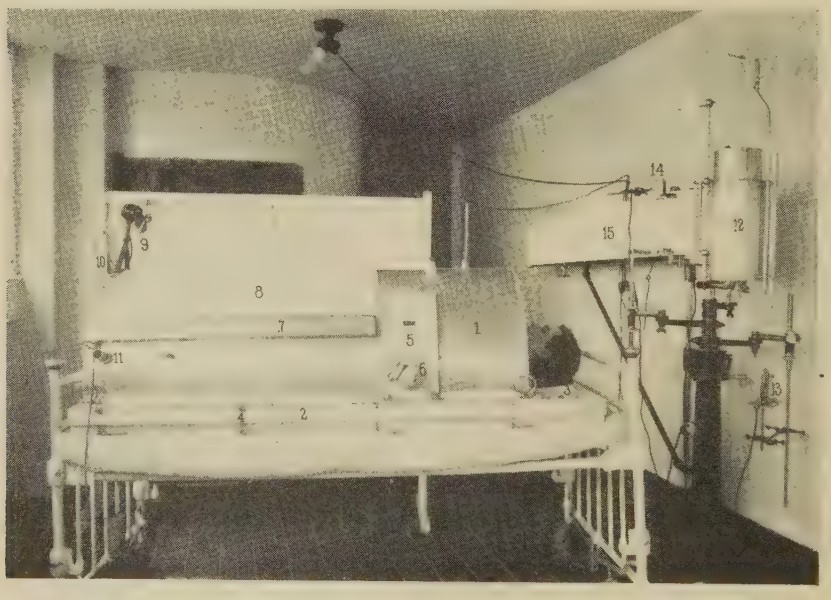

FIG. 1.

1. Plethysmograph.
2. Oak base.
3. Adjustable head rest.
4. Eccentric clamp.
5. Door for access to patient.
6. Arm hole.
7. Trough containing water.
8. Moistened cloth.
9. Fan.
10. Housing containing relay and thermostat contacts. (The toluol tube projects into the plethysmograph for a distance of 35 cm.)
11. Hose connection to communicate with volume recorder.
12. Volume recorder.
13. Work adder.
14. Signal lever activated by each complete revolution of work adder wheel.
15. Kymograph.

NOTE: The apparatus shown in the figure was made by Mr. Joseph Becker, 437 West 59th Street, New York City. The authors wish to thank him for his skill and helpful ingenuity.

against the rim of the opening. The general plan of the plethysmograph is shown in Figure 1.

Temperature Control. Without some provision for temperature control the air surrounding the subject will be gradually warmed by the heat given off from his body. This may cause a rise of temperature within the plethysmograph of 2° C. in 1 hr. (Table I), or more in the case of a febrile patient. A simple method of cooling was devised by playing a current of air from an electric fan on to the surface of the plethysmograph previously covered by a moistened cloth. The fan is automatically operated by a relay which in turn is activated by a toluol-mercury thermostat, the bulb of which lies within the plethysmograph. To keep the cloth moist, its longitudinal edges are allowed to dip into 2 water-containing troughs fixed to either side of the aluminum tunnel. With this arrangement the temperature within the plethysmograph can be kept fairly constant, the fluctuations usually not exceeding 0.5° C.

TABLE I.

Showing increase of temperature inside Plethysmograph when no cooling device is used.

Time	Room Temperature	Plethysmograph temperature
11:20	23.0	24.1
11:30	Subject introduced into plethysmograph	
11:35	22.7	27.0
11:40	22.6	27.5
11:50	22.6	28.0
12:03	22.5	28.1
12:12	22.6	28.6
12:23	22.5	28.9
12:33	22.7	29.0

TABLE II.

Showing relative constant temperature inside Plethysmograph when thermostatically controlled fan is used.

Time	Room Temperature	Plethysmograph temperature
11:10	21.2	25.0
11:18	21.3	25.2
11:30	21.1	25.2
11:45	21.2	25.1
11:55	20.8	25.2
12:05	20.7	25.0

Recording Device. For purposes of registering graphically the respiratory movements, the plethysmograph is made to communicate by means of a wide bore rubber tubing with a sensitive, well counterpoised spirometer. The movements of the spirometer bell are recorded in the usual manner on a kymograph. To obviate the

necessity of separate measurements of each line in the tracing the spirometer bell is connected with a work adder which integrates the excursions of the volume recorder. Each complete revolution of the work adder wheel is graphically recorded by a signal lever electrically through a relay. One revolution is equivalent to an air flow of 12.34 liters.

To make sure that the apparatus is leak-tight it is necessary only to place a 10 gm. weight on the top of the spirometer bell. If the level of the bell does not progressively fall there is no leak.

Use of Apparatus. The apparatus here described is at present being used for the quantitative study of the respiratory movements in patients suffering from pneumonia. An attempt is being made to correlate the condition of oxygen want, as measured by the percentage saturation of the arterial blood, with the rate and depth of the respiratory movements. The effect of morphine and oxygen on the state of anoxemia and on the character of the breathing is being studied.

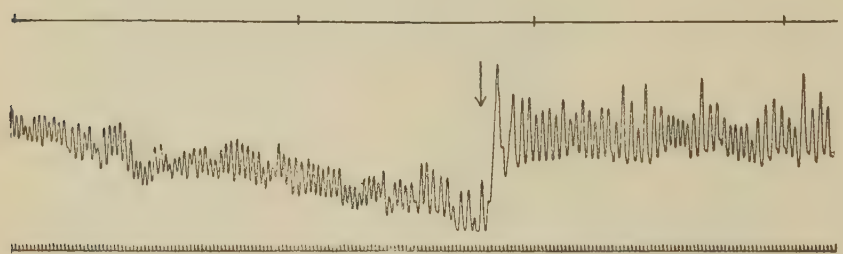

FIG. 2.

Graphic tracing showing the effect of applying a respiratory mask lightly to the face. The arrow indicates the point at which the mask was put into position.

Upper line traced by work adder signal lever. The distance between marks records an air flow of 12.34 liters.

Lower line records time in 2 second intervals.

To show the artificial conditions imposed by the use of a mask, a graphic record is reproduced in Fig. 2. The first part of the record shows rate and depth of breathing when the patient in the plethysmograph was breathing naturally. In the second part of the record a light metal face mask with pneumatic rubber gasket was fitted over the patient's nose and mouth. There resulted an immediate change in the character of the breathing.

3975

The Identity of C. Oedematoides and B. Sordellii.

FREDERICK HUMPHREYS AND FRANK L. MELENEY.

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In 1927, we, with Karp,[1] reported the isolation from a fatal human case of "gas gangrene" of a pathogenic anaerobic organism which did not correspond either culturally or immunologically to any of the hitherto well recognized clostridia; and, shortly after, we were able to isolate the same organism from specimens of imperfectly sterilized cat-gut.[2] This organism somewhat resembled Weinberg's *B. oedematiens* culturally. When injected into mice subcutaneously it produced much the same type of lesion, *i. e.*, the white gelatinous oedema so characteristic of the latter organism.

It differed from *B. oedematiens,* however, in failing to ferment glycerol; it was more actively proteolytic; and, more especially, the toxin readily obtained by filtering cultures, was in no way neutralized by high-titre anti-oedematiens serum. On the other hand, anti-serum prepared by the immunization of a rabbit with this sterile filtrate afforded full protection against the homologous toxin, but not at all against the toxins of *B. welchii,* of *vibrion septique* or of *B. oedematiens.*

For these reasons we concluded that the organism should be recognized as a distinct species and suggested the name *Clostridium oedematoides.*

A short time later, Hall and Scott[3] published a restudy of 2 strains of organisms sent to them from South America by Sordellii, which the latter had described first in 1922[4] and had named *B. oedematis sporogenes.* Although Hall and Scott found one of these strains to be entirely non-pathogenic for laboratory animals, they found that the other was fully virulent and that it produced a potent exo-toxin which could be separated from cultures by filtration. Hall and Scott suggested that the original name, *B. oedematis sporogenes* should be replaced by the binomial *B. sordellii.* They also suggested that the cultural characteristics of Sordellii's organism were so similar to

1 PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 675.

2 *Surg. Gyn. and Obstet.,* 1927, 775.

3 *J. Infect. Dis.,* xli, 329.

4 *C. R. Soc. de Biol.,* lxxxvii, 838; lxxxix, 53; xci, 1033.

those of *C. oedematoides* as described by us, as to render it probable that the 2 species were identical. They did not, however, carry out any immunologic or specific-protection experiments.

It is our conviction that, in the case of the pathogenic clostridia at any rate, specific toxin-antitoxin experiments, where applicable, should be the final court of appeal in determining the identity or non-identity of two possibly different species.

Minor cultural characteristics, details of colony configuration and of individual morphology, etc., often show such variation even within the same strain under slightly different conditions of growth, that they afford unsafe criteria. Again, agglutination reactions in this group are notoriously unreliable owing on the one hand to the indefinite number of sub-groups liable to be encountered, and, on the other hand, to their frequent tendency to spontaneous agglutination.

It seemed necessary to settle the question: Does antitoxin prepared against the toxin of *B. sordellii* protect against the toxin of *C. oedematoides;* and, conversely, does the antitoxin prepared against the toxin of *C. oedematoides* protect against the toxin of *B. sordellii?*

Strains of *B. sordellii* were kindly furnished us by Professor Hall. Rather casual cultural comparison of the 2 organisms revealed no fundamental differences. The surface colonies on anaerobic blood agar plates were very similar, though *B. sordellii* was slightly more hemolytic than the other. Correlated with this, perhaps, it was found to be somewhat more powerfully proteolytic when growing on the surface of a Loeffler coagulated serum slant. No constant morphological differences were noted. Both organisms made gas and acid from dextrose but not from lactose, saccharose, salicin or glycerol.

A toxin of *B. sordellii* was prepared by heavily seeding a flask of cooked meat broth (1% Wittes peptone) to which 0.2% dextrose had been added. This was incubated for 40 hours in a modification of the McIntosh and Filds anaerobic jar, strained through glass wool, centrifugalized until clear, and finally sucked rapidly through a Berkefeld N filter. The resultant fluid was sterile, clear and slightly acid (pH 6.0). Injected subcutaneously into mice in doses of 0.02 mil, it caused death in 48 hours with slight gelatinous oedema. Preserved unsealed, even on ice, the titre fell off rapidly, 0.075 mils failing to kill 1 week later; when sealed with sterile vaseline, however, the titre was maintained fairly well.

Attempts were then made to immunize rabbits to the filtrate. Rabbits appear to be peculiarly susceptible to the toxin; and several

animals, apparently well on their way toward immunization were lost, owing either to too rapidly increasing dosage or to too short intervals between injections. But finally by starting with doses of 0.05 mils and injecting intravenously at weekly intervals, covering in all a period of 2 months, very gradually increasing the amounts, we obtained an animal that could withstand 1.5 mils without symptoms and whose serum was distinctly antitoxic. Eleven days after this last injection of 1.5 mils, the rabbit was bled to death.

Meanwhile a fresh filtrate from our *C. oedematoides* was prepared in exactly the same manner as described above. It resembled that obtained from *B. sordellii* in every way except that it was somewhat less acid (pH 7.0). The M.L.D. for mice was also 0.02 mil.

For oedematoides antitoxin we used some of the serum that had been made the previous year for our original experiments and which was still sufficiently potent.

Twenty gm. mice were injected intraperitoneally with mixtures of serum and filtrates as indicated below. The mixtures were incubated in the water-bath at 37° C. for one half hour before injecting.

Mouse No. 1—0.2 mil. *oedematoides* serum + 0.2 *oedematoides* filtrate. Result: No symptoms. Survived indefinitely.

Mouse No. 2—0.2 mil *B. sordellii* serum + 0.2 mil *oedematoides* filtrate. Result: No symptoms. Survived indefinitely.

Mouse No. 3—0.2 mil normal rabbit serum + 0.2 mil *oedematoides* filtrate. Result: Died within 24 hours.

Mouse No. 4—0.2 mil Saline + 0.2 mil *oedematoides* filtrate. Result: Died within 24 hours.

Mouse No. 5—0.2 mil *oedematoides* serum + 0.2 mil *B. sordellii* filtrate. Result: No symptoms. Survived indefinitely.

Mouse No. 6—0.2 mil *B. sordellii* serum + 0.2 mil *B. sordellii* filtrate. Result: No symptoms. Survived indefinitely.

Mouse No. 7—0.2 mil normal rabbit serum + 0.2 mil *B. sordellii* filtrate. Result: Died within 24 hours.

Mouse No. 8—0.2 mil Saline + 0.2 mil *B. sordellii* filtrate. Result: Died within 24 hours.

Thus it is shown that 0.2 mil of either *B. sordellii* or *C. oedematoides* antitoxin protects against 10 M.L.D. of the toxic filtrate of either organism, whereas normal serum has no such action.

Conclusion: Clostridium oedematoides as described by Meleney, Humphreys and Carp is identical to Sordelli's *B. oedematis sporogenes,* redescribed by Hall and Scott as *B. sordellii.*

To Sordellii is due the credit of priority, although our study of the organism was made independently and without knowledge of his work. The occurrence of Sordellii's strains and ours in such widely separated places is of interest.